Novel nervous and multi-system regenerative therapeutic strategies for diabetes mellitus with mTOR

Kenneth Maiese*

Cellular and Molecular Signaling, Newark, NJ, USA

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Abstract
Throughout the globe, diabetes mellitus (DM) is increasing in incidence with limited therapies presently available to prevent or resolve the significant complications of this disorder. DM impacts multiple organs and affects all components of the central and peripheral nervous systems that can range from dementia to diabetic neuropathy. The mechanistic target of rapamycin (mTOR) is a promising agent for the development of novel regenerative strategies for the treatment of DM. mTOR and its related signaling pathways impact multiple metabolic parameters that include cellular metabolic homeostasis, insulin resistance, insulin secretion, stem cell proliferation and differentiation, pancreatic β-cell function, and programmed cell death with apoptosis and autophagy. mTOR is central element for the protein complexes mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) and is a critical component for a number of signaling pathways that involve phosphoinositide 3-kinase (PI 3-K), protein kinase B (Akt), AMP activated protein kinase (AMPK), silent mating type information regulation 2 homolog 1 (Saccharomyces cerevisiae) (SIRT1), Wnt1 inducible signaling pathway protein 1 (WISP1), and growth factors. As a result, mTOR represents an exciting target to offer new clinical avenues for the treatment of DM and the complications of this disease. Future studies directed to elucidate the delicate balance mTOR holds over cellular metabolism and the impact of its broad signaling pathways should foster the translation of these targets into effective clinical regimens for DM.

Key Words: Akt; AMP activated protein kinase (AMPK); apoptosis; Alzheimer’s disease; autophagy; β-cell; cancer; cardiovascular disease; caspase; CCN family; diabetes mellitus; epidermal growth factor; erythropoietin; fibroblast growth factor; forkhead transcription factors; FoxO; FRAP1; hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) (TSC1/TSC2); insulin; mechanistic target of rapamycin (mTOR); mTOR Complex 1 (mTORC1); mTOR Complex 2 (mTORC2); nicotinamide; nicotinamide adenine dinucleotide (NAD+); non-communicable diseases; oxidative stress; phosphoinositide 3-kinase (PI 3-K); programmed cell death; silent mating type information regulation 2 homolog 1 (Saccharomyces cerevisiae) (SIRT1); sirtuin; stem cells; wingless; Wnt; Wnt1 inducible signaling pathway protein 1 (WISP1)

Introduction
The incidence of non-communicable diseases (NCDs) is increasing throughout the world. According to the World Health Organization, greater than 60 percent of the 57 million global deaths are attributable to NCDs (World Health Organization, 2011). In low and middle-income countries, NCDs can affect almost one-third of the population under the age of 60. Conversely, slightly greater than 10 percent of the population under 60 is affected in high-income countries (World Health Organization, 2011). The rise in NCDs parallels the increase in life expectancy of the world’s population. Improvements in effective treatments for multiple disorders and broader access to preventive care have most likely contributed to the increased life span of the global population. For example, the number of individuals over the age of 65 has doubled during the previous 50 years with life expectancy approaching 80 years of age. In addition, life expectancy has been marked by a 1 percent decrease in the age-adjusted death rate from the years 2000 through 2011 (Minino, 2013).

One of the most significant NCDs that affect the global population is diabetes mellitus (DM) (Haldar et al., 2015; Maiese, 2015h). DM is increasing in incidence throughout the world. It is estimated that approximately 350 million individuals currently have DM (Maiese et al., 2011, 2013a; Rutter et al., 2012; Jia et al., 2014; Xu et al., 2014a) and another 8 million individuals are believed to suffer from metabolic disorders but remain undiagnosed at present (Harris and Eastman, 2000; Maiese et al., 2007; Maiese, 2015f). Financial costs for DM are also significant. In the United States (US), almost 9,000 US dollars are required to care for each individual with DM per year. The care for patients with DM consumes 17 percent of the Gross Domestic Product in the US as reported by the Centers for Medicare and Medicaid Services (CMS) (Centers for Medicare and Medicaid Services, 2013). Approximately $176 billion is required for direct medical costs and another $69 billion in lost finances...
results from reduced productivity tied to DM.

Obesity and impaired glucose tolerance further complicate the clinical presentation for DM (Maiiese et al., 2015e; Tuulsilak et al., 2016). Impaired glucose tolerance in the young as well as the presence of obesity increases the risk of developing DM in these individuals (Maiiese et al., 2011). Obesity and excess body fat can lead to alterations in protein tyrosine phosphatase signaling, insulin resistance, oxidative stress mediated cell death, cellular inflammation, mitochondrial dysfunction, impairments in growth factor function, injury to pancreatic β cells, and altered DNA methylation (Xu et al., 2014a,b; Maiiese, 2015c; Mikhe et al., 2015; Snyder and Stefano, 2015; Wang et al., 2015; Xiao et al., 2015).

Early diagnosis of DM and quickly instituting available therapies for individuals with DM can offer some degree of improvement and slow the progression of DM. However, tight serum glucose control does not always lead to the resolution of complications from DM (Maiiese et al., 2011; Coca et al., 2012). Use of diet control treatments may be effective to prevent hyperglycemic events, but these strategies also can potentially decrease organ mass through processes that involve autophagy (Lee et al., 2014).

DM can be classified as either non-insulin dependent (Type 1) DM or insulin dependent (Type 2) DM (Maiiese et al., 2010, 2013b). Type 1 DM occurs in ten percent of patients. It is an autoimmune disorder associated with the alleles of the human leukocyte antigen class II genes within the major histocompatibility complex (Maiiese et al., 2007). Insulin production and homeostasis is lost with the destruction of pancreatic β-cells with inflammatory infiltration of the islets of Langerhans. Almost ninety percent of patients with Type 1 DM have increased titters of autoantibodies (Type 1A DM), but the remaining ten percent of Type 1 DM individuals do not have these serum autoantibodies (Maiiese, 2015f, h). These individuals have maturity-onset diabetes of the young (MODY) that can occur form the β-cell dysfunction with autosomal-dominant inheritance (Type IB DM). Type 2 DM is present in ninety percent of individuals and usually occurs in individuals over the age of 40. A progressive deterioration of glucose tolerance with early β-cell compensation results with Type 2 DM (Maiiese et al., 2007, 2013a). Loss of insulin secretion is a result of multiple factors that involve prolonged exposure to free fatty acids and hyperglycemia, impaired β-cell function, and the absence of inhibitory feedback through plasma glucagon levels. Type 1 and Type 2 DM have functional overlap. Approximately ten percent of individuals with Type 2 DM can have elevated serum autoantibodies similar to Type 1 DM. Insulin resistance also may exist in some patients with Type 1 DM (Maiiese, 2015e).

DM is a multi-system disease that can lead to progressive deterioration of the body (Esser et al., 2015; Gomez-Brouchet et al., 2015; Haldar et al., 2015; Maiiese, 2015f). For example, DM can affect the nervous system and lead to peripheral nerve disorders, cognitive loss that also may be associated with Alzheimer’s disease (AD) (Maiiese et al., 2008a; Du et al., 2015; Kapogiannis et al., 2015; White, 2014), loss of neuronal cell longevity (White, 2014), psychiatric disorders (Hadamitzky et al., 2014; Ignacio et al., 2015), visual impairment (Fu et al., 2012; Lee et al., 2012a; Busch et al., 2014; Maiiese, 2015f), and stroke (Maiiese et al., 2008b; Alexandru et al., 2012; Jiang et al., 2014; Xu et al., 2014b; Maiiese, 2015a; Xiao et al., 2015). In the central nervous system, insulin resistance and dementia that occur during DM has been shown to be present in patients with Alzheimer’s disease (Maiiese et al., 2007; Sonnen et al., 2009), demonstrating that degeneration in the nervous system may be the result of impaired cellular metabolism similar to that which occurs during DM (Kapogiannis et al., 2015). In the peripheral nervous system, DM can lead to autonomic neuropathy (Albiero et al., 2014) and peripheral nerve disease (Gomes and Negrato, 2014; Gomez-Brouchet et al., 2015). It is estimated that at least seventy percent of individuals with DM can develop some degree of diabetic peripheral neuropathy. Assessment of the course of the disease may be difficult since the disorder is chronic in nature, may be sub-clinical, and prior deficits may go undetected if improved control over glucose homeostasis is initiated. Closely tied to neuronal degeneration is the loss of the neurovascular unit. DM can lead to impairment of the neuroglialvascular unit (Busch et al., 2014), endothelial cell injury (Chong et al., 2007, 2011; Hou et al., 2010a; Schaffer et al., 2012; Liu et al., 2013b; Wang et al., 2014a; Zhang et al., 2014), loss of angiogenesis (Chen et al., 2012), endothelial cell senescence (Arunachalam et al., 2014), and cardiovascular complications (Chong and Maiiese, 2012; Xu et al., 2014b; Yu et al., 2015a). DM also can have detrimental effects on the immune system, musculoskeletal function, hepatic metabolism, and renal clearance (D’Onofrio et al., 2015; Esser et al., 2015).

Given the significant impact DM has only multiple systems of the body and especially the nervous system, new therapeutic strategies that can address the onset and progression of DM in the body are desperately needed. In particular, the mechanistic target of rapamycin (mTOR) is one such avenue to consider the development of novel effective strategies to repair and potentially regenerate injured portions of the nervous system during DM. mTOR is tightly linked to DM cellular metabolism (Maiiese et al., 2013c; Maiiese, 2014b; Johnson et al., 2015). For example, mTOR can lead to pancreatic β-cell proliferation (Miao et al., 2013), block neuronal cell apoptosis during DM through the epidermal growth factor (EGF) receptor (Kimura et al., 2013), stimulate adipocyte differentiation to enhance glucose uptake (Jung et al., 2015), may protect against cognitive loss during DM through increased expression of acetylcholinesterase (AChE) (Liu et al., 2015), and can foster glucose homeostasis (Malla et al., 2015a).

mTOR Signaling in Metabolic Disease

mTOR, a 289-kDa serine/threonine protein kinase that is encoded by a single gene FRAP1, represents one such target for novel strategies of drug development for the treatment of DM and the complications of this disorder (Chong and Maiiese, 2012; Zhou et al., 2015; Berry et al., 2016). mTOR also is recognized as the mammalian target of rapamycin and the FK506-binding protein 12-rapamycin complex-associated protein 1. The target of rapamycin (TOR) was initially
described in *Saccharomyces cerevisiae* with the genes TOR1 and TOR2 (Maiese et al., 2013c). Through the use of rapamycin-resistant TOR mutants, TOR1 and TOR2 were found to encode the Tor1 and Tor2 isoforms in yeast (Heitman et al., 1991). Rapamycin is a macrolide antibiotic (Singh et al., 1979) in *Streptomyces hygroscopicus* that blocks TOR and mTOR activity (Maiese, 2015j). mTOR forms the principal component of the protein complexes mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) (Figure 1) (Gulhati et al., 2011; Zoncu et al., 2011; Chong et al., 2012c; Maiese, 2014d). Rapamycin can prevent mTORC1 activity by binding to immunophilin FK-506-binding protein 12 (FKBP12) that attaches to the FKBP12 -rapamycin-binding domain (FRB) at the carboxy (C) -terminal of mTOR to interfere with the FRB domain of mTORC1. The precise mechanism of how rapamycin interaction with the domain of FRB leads to inhibition of mTORC1 is unclear, but may involve allosteric changes on the catalytic domain as well as the inhibition of phosphorylation of protein kinase B (Akt) and p70 ribosomal S6 kinase (p70S6K) (Xue et al., 2009). In general, mTORC1 is more sensitive to inhibition by rapamycin than mTORC2, but chronic administration of rapamycin can inhibit mTORC2 activity as a result of the disruption of the assembly of mTORC2 (Sarbasov et al., 2006). Overall, mTOR is ubiquitous in the body and drives gene transcription, protein formation, cellular proliferation, and the metabolic function of cells.

mTORC1 consists of Raptor, the proline rich Akt substrate 40 kDa (PRAS40), Deptor (DEP domain-containing mammalian lethal with Sec13 protein 8, termed mLST8) (mLST8/G L) (Figure 1) (Maiese et al., 2013c). mTORC1 can bind to its constituents through the protein Ras homologue enriched in brain (Rheb) that phosphorylates the Raptor residue serine895 and other residues that include serine896, serine183, serine1981, and threonine1986 (Foster et al., 2010). The inability to phosphorylate serine895 limits mTORC1 activity, as demonstrated using a site-direct mutation of serine895 (Wang et al., 2009). In this system, mTOR can control Raptor activity that can be blocked by rapamycin (Wang et al., 2009). Deptor also is inhibitory. Deptor blocks mTORC1 activity by binding to the FAT (FKBP12-rapamycin-associated protein (FRAP), ataxia-telangiectasia (ATM), and the transactivation/transformation domain-associated protein) domain of mTOR. If the activity of Deptor is diminished, protein kinase B (Akt), mTORC1, and mTORC2 activity are increased (Peterson et al., 2009). PRAS40 also can inhibit mTORC1 activity. PRAS40 blocks mTORC1 activity by preventing the association of p70 ribosomal S6 kinase (p70S6K) and the eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) with Raptor (Maiese, 2014b; Malla et al., 2015b). mTORC1 becomes active once Akt phosphorylates PRAS40. This releases PRAS40 from Raptor to sequester PRAS40 in the cytoplasm with the docking protein 14-3-3 (Fonseca et al., 2007; Chong et al., 2012b; Shang et al., 2012; Wang et al., 2012a; Xiong et al., 2014). In contrast to Deptor and PRAS40, mLST8 fosters mTOR kinase activity. This involves the binding of p70S6K and 4EBP1 to Raptor (Kim et al., 2003). mLST8 also controls insulin signaling through the transcription factor FoxO3 (Guertin et al., 2006; Maiese, 2015i), is necessary for Akt and protein kinase C-α (PKCα) phosphorylation, and is required for Rictor to associate with mTOR (Guertin et al., 2006).

In relation to mTORC2 (Figure 1), mTORC2 consists of Rictor, mLST8, Deptor, the mammalian stress-activated protein kinase interacting protein (mSIN1), and the protein observed with Rictor-1 (Protor-1) (Chong et al., 2010; Gliddon et al., 2012; James et al., 2012; Kamarudin et al., 2014; Maiese, 2014a; Tang et al., 2014). mTORC2 is involved in cytoskeleton remodeling through PKCα and cell migration through the Rac guanine nucleotide exchange factors P-Rex1 and P-Rex2 and through Rho signaling (Jacinto et al., 2004). mTORC2 may be necessary to maintain glucose homeostasis, since loss of this pathway can promote sever hyperglycemia (Treins et al., 2012). Impairment in mTORC2 signaling also leads to oxidative damage and insulin resistance (Wang et al., 2011a). In addition, mTORC2 signaling plays a significant role for the maintenance of pancreatic β-cell proliferation and mass (Gu et al., 2011).

mTORC2 can activate protein kinases, such as glucocorticoid induced protein kinase 1 (SGK1), a member of the protein kinase A/protein kinase G/protein kinase C (AGC) family of protein kinases. Protor-1, a Rictor-binding subunit of mTORC2, activates SGK1 (Garcia-Martinez and Alessi, 2008; Pearce et al., 2011). The kinase domain of mTOR can phosphorylate mSIN1 and prevent lysosomal degradation of this protein. Rictor (Sarbasov et al., 2005) and mSIN1 (Frias et al., 2006) also can phosphorylate Akt at serine473 and foster threonine468 phosphorylation by phosphoinositide-dependant kinase 1 (PDK1) to enhance cell survival.

Phosphoinositide 3-kinase (PI 3-K) and Akt are critical in mTOR signaling (Neasta et al., 2014; Korpi et al., 2015; Liu et al., 2015; Moon et al., 2015; Sun et al., 2015) (Figure 2). The terminal domains of mTOR control the catalytic activity, binding, and phosphorylation of mTOR (Maiese et al., 2013c). In particular, the C-terminal domain of mTOR possesses a sequence homology to the catalytic domain of the PI 3-K family and contains several phosphorylation sites that regulate mTOR. Downstream from PI 3-K, Akt can block activity of the hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) (TSC1/TSC2) complex that inhibits mTORC1 (Chong et al., 2012a; Janku et al., 2012; Maiese, 2013; Morgan-Warren et al., 2013). Control of the TSC1/TSC2 complex is principally controlled through Akt and its phosphorylation of TSC2 (Maiese, 2014a). Extracellular signal-regulated kinases (ERKs), protein p90 ribosomal S6 kinase 1 (RSK1), glycogen synthase kinase 3β (GSK-3β), and AMP activated protein kinase (AMPK) also can modulate the activity TSC1/TSC2 complex. Akt can phosphorylate TSC2 at serine1439, serine1981, and threonine1462 to lead to the binding of TSC2 to cytoplasmic protein 14-3-3, disengagement of the TSC1/TSC2 complex, and activation of Rho and mTORC1 (Cai et al., 2006). However under some conditions that promote cell survival, a limited activity of TSC2 and AMPK is necessary since complete knockdown of TSC2 can result in cell death (Shang et al., 2013). In contrast to the TSC1/TSC2 complex inhibiting mTORC1 activity, mTORC2 activity is increased during activation of the TSC1/TSC2 complex through the amino (N)-terminal region of TSC2 and the C-terminal region of Rictor (Huang et al., 2008).
Similar to PI 3-K and Akt, AMPK plays a significant role in the control of mTORC1 activity, especially in metabolic disease (Figure 2). AMPK can inhibit mTORC1 activity through the activation of the TSC1/TSC2 complex. TSC2 functions as a GTPase-activating protein (GAP) converting G protein Rheb (Rheb-GTP) into the inactive GDP-bound form (Rheb-GDP). Once Rheb-GTP is active, Rheb-GTP associates with Raptor to oversee the binding of 4EBP1 to mTORC1 and increase mTORC1 activity (Sato et al., 2009). AMPK phosphorylates TSC2 to increase GAP activity to change Rheb-GTP into the inactive Rheb-GDP and to block mTORC1 activity (Inoki et al., 2003). AMPK also can increase RTP801 (REDD1/ product of the Ddit4 gene) expression, an inhibitor of mTOR signaling (Benyoucef et al., 2015), to increase TSC1/TSC2 activity and suppress mTORC1 activity by releasing TSC2 from its association with protein 14-3-3. AMPK interfaces with other cellular pathways that can affect cell survival and stem cell maintenance, such as the silent mating type information regulation 2 homolog 1 (Saccharomyces cerevisiae) (SIRT1) (Favoro et al., 2015; Maiese, 2015a). AMPK increases nicotinamide phosphoribosyltransferase (NAMPT) activity to convert nicotinamide to nicotinamide mononucleotide (NAD+) (Wang et al., 2014b; Maiese, 2015k). As a result, this increases nicotinamide adenine dinucleotide (NAD+) levels, decreases levels of the SIRT1 inhibitor nicotinamide, and promotes SIRT1 transcription (Wang et al., 2011b; Chong et al., 2012d; Maiese, 2015h). With an increased intracellular NAD+/NADH ratio, AMPK deacetylates the SIRT1 targets peroxisome proliferator-activated receptor-gamma coactivator 1 (PGC-1β) and forkhead transcription factors that include FoxO1 (Maiese et al., 2009a) and FoxO3a (Canto and Auwerx, 2009). Together, SIRT1 and AMPK can function as inhibitors of mTOR (Maiese et al., 2013a).

In regards to metabolic disease, AMPK has been shown to reduce insulin resistance, since the loss of AMPK results in reduced tolerance to the development of insulin resistance (Liu et al., 2014b). AMPK activation may improve memory retention in models of AD and DM (Du et al., 2015), maintain the proper metabolic function of cells, prevent adipocyte differentiation, lipid accumulation, and obesity, and limit cardiac ischemia in animal models (Maiese, 2015k).

In addition, metformin, an agent that controls hyperglycemia in DM, inhibits mTOR activity and leads to the induction of autophagy. Metformin can activate AMPK (Leclerc et al., 2013) and also block mTOR activity through pathways independent of AMPK (Kalender et al., 2010). Metformin prevents cell loss during hypoxia through increased AMPK activity (Sheng et al., 2012), confers neuroprotection (Jiang et al., 2014), reduces cardiomyopathy in experimental models of DM (Xie et al., 2011) and prevents endothelial cell senescence (Arunachalam et al., 2014). With SIRT1, AMPK inhibits mTORC1 activity and SIRT1 assists with mesangial cell proliferation during high glucose exposure (Zhang et al., 2012). In combination, SIRT1 and AMPK may offer cellular protection of endothelial cells through the induction of autophagy against oxidized low-density lipoproteins (Jin et al., 2014). However, under some conditions, limited AMPK activity may be better suited for cellular protection in DM.

Reduced AMPK activity can promote the protection of pancreatic islet cells in mice (Guan et al., 2014), limit amyloid (Aβ) toxicity (Shang et al., 2013), and prevent inflammation in the nervous system (Russo et al., 2014).

**mTOR and Programmed Cell Death**

For mTOR to control the survival of cells during DM, mTOR must oversee the programmed cell death pathways of apoptosis and autophagy (Figure 1) (Maiese et al., 2012b). Apoptosis consists of a cascade that activates nuclease and proteases involving caspases that can affect both the early phase of apoptosis with the loss of plasma membrane phosphatidylserine (PS) asymmetry and a later phase that leads to genomic DNA degradation (Shang et al., 2010; Viola et al., 2012; Wong et al., 2012). Membrane PS externalization, a reversible process, activates inflammatory cells to engulf and remove injured cells (Shang et al., 2009; Bailey et al., 2010; Hou et al., 2010b; Wei et al., 2013). If this process is prevented, loss of functional cells expressing membrane PS residues can be averted and genomic DNA degradation does not result (Yang et al., 2013; Weinberg et al., 2014; Maiese, 2015e). The destruction of cellular DNA is not a reversible process (Kim et al., 2015; Maiese, 2015f; Xin et al., 2015; Yu et al., 2015b).

During DM, apoptosis affects multiple cell types leading to cell death in endothelial cells, renal cells, neurons, cardiomyocytes, and pancreatic β-cells. In addition, DM can incite multiple pathways of programmed cell death. “Highly-oxidized glycated” low-density lipoproteins that are formed during DM lead to oxidative stress in human retinal capillary pericytes with subsequent induction of both apoptosis and autophagy (Fu et al., 2012). Impairment of mitochondrial dysfunction that can occur during DM and oxidative stress also can affect the induction of apoptotic pathways (Perez-Gallardo et al., 2014; Maiese, 2015k; Mikhed et al., 2015; Parmar et al., 2015; Wang et al., 2015). Mitochondrial dysfunction results in the opening of the mitochondrial membrane permeability transition pore, release of cytochrome c, and caspase activation (Maiese et al., 2010; Perez-Gallardo et al., 2014; Wang et al., 2014a; Poulouse and Raju, 2015). Glucolipotoxicity exposure to pancreatic β-cells results in oxidative stress and mitochondrial dysfunction with cytochrome c release, caspase activation, and apoptosis (Liu et al., 2012). In addition, loss of functional mitochondrial proteins and mitochondrial DNA in adipocytes has been associated with the development of Type 2 DM (Choo et al., 2006). Patients with Type 2 DM also have impaired skeletal muscle mitochondrial activity than those in control subjects (Petersen et al., 2004; Newsholme et al., 2012).

mTOR activation blocks apoptosis to limit insulin resistance and vascular thrombosis in patients with metabolic syndrome (Figure 1). Increased activity of mTOR also may prevent the development of atherosclerosis (Peng et al., 2014). mTOR activation through glucagon-like peptide-1 agonists can protect pancreatic β-cells from cholesterol mediated apoptotic cell injury (Zhou et al., 2015), prevent neural apoptotic cell loss during DM through the epidermal growth factor receptor (EGF) (Kimura et al., 2013), and foster pancreatic β-cell proliferation (Miao et al., 2013) (Figure 2).
Figure 1 mTOR controls the structure and function of mTORC1 and mTORC2 to ultimately impact programmed cell death in diabetes mellitus through apoptosis and autophagy.

The mechanistic target of rapamycin (mTOR) is a critical element of both mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2). mTORC1 is composed of mTOR, Raptor (Regulatory-Associated Protein of mTOR), the proline rich Akt substrate 40 kDa (PRAS40), the mammalian lethal with Sec13 protein 8 (mLST8/G L), and Deptor (DEP domain-containing mTOR interacting protein). mTORC2 consists of mTOR, Rictor (Rapamycin-Insensitive Companion of mTOR), the mammalian stress-activated protein kinase interacting protein (mSin1), the protein observed with Rictor-1 (Protor-1), Deptor, and mLST8. Activation of mTOR can block apoptotic cell injury while inhibition of mTOR can lead to the induction of autophagy that affects insulin resistance and inflammation.

During periods of reduced mTOR activity, induction of autophagy can result that can be either beneficial or detrimental during DM (Figure 1) (Maiese, 2015b). At least 33 autophagic related genes (Atg) that have been identified in yeast with TOR can affect multiple disorders including DM (Weckman et al., 2014; Maiese, 2015k). Atg1, Atg13 (also known as App13), and Atg17 are associated with the PI 3-K, Akt, and TOR pathways (Klionsky et al., 2016). Either the Atg1 complex in yeast or the UNC-51 like kinase 1 (ULK1) complex in mammals is necessary for the induction of autophagy (Kamada et al., 2000; Maiese et al., 2012b). The mammalian homologues of Atg1 are UNC-51 like kinase 1 (ULK1) and ULK2 (Chong et al., 2012c). Mammalian Atg13 binds to ULK1, ULK2, and FIP200 (focal adhesion kinase family interacting protein of 200 kDa) to activate ULKS, promote the phosphorylation of FIP200 by ULKS, and lead to autophagy induction (Jung et al., 2009). mTOR activity blocks the onset of autophagy by phosphorylating Atg13 and ULKS to prevent formation of the ULK-Atg13-FIP200 complex (Jung et al., 2009). In general, autophagy recycles components of the cell cytoplasm to remove non-functional organelles and initiate tissue remodeling (Maiese et al., 2012b; Francois et al., 2014; Vakifahmetoglu-Norberg et al., 2015; Nakka et al., 2016). Macroautophagy is the classification of autophagy most often responsible for the recycling of organelles and consists of the sequestration of cytoplasmic proteins and organelles into autophagosomes that combine with lysosomes for degradation and recycling (Maiese, 2014b; Frederick et al., 2015; Sasazawa et al., 2015). Other categories for autophagy include microautophagy that uses the invagination of the lysosomal membrane for the sequestration and digestion of cytoplasmic components (Maiese et al., 2012b). Another category, chaperone-mediated autophagy, uses cytosolic chaperones to transport cytoplasmic components across lysosomal membranes (Maiese, 2015i).

Figure 2 mTOR signaling in diabetes mellitus is intimately tied to several critical pathways that can govern cellular metabolism. The mTOR signaling axis relies upon phosphoinositide 3-kinase (PI 3-K), protein kinase B (Akt), AMP activated protein kinase (AMPK), silent mating type information regulation 2 homolog 1 (Saccharomyces cerevisiae) (SIRT1), Wnt1 inducible signaling pathway protein 1 (WISPI), and the growth factors epidermal growth factor (EGF), fibroblast growth factor (FGF), and erythropoietin (EPO) to regulate metabolic pathways. mTOR and its related signaling pathways can oversee metabolic homeostasis, stem cell maintenance and viability, insulin secretion and resistance, apoptosis, autophagy, and pancreatic β-cell mass and function.

However, induction of autophagy may be harmful even during periods when it is not the primary determinant of
cell injury, such as during apoptotic cell death (Wang et al., 2012b). Heightened activity of autophagy can lead to significant loss of cardiac and liver tissue in diabetic rats during attempts to achieve glycemic control through diet modification (Lee et al., 2014). During periods of elevated glucose that occur in DM, advanced glycation end products (AGEs), agents that can result in complications during DM, have been shown to lead to the induction of autophagy and vascular smooth muscle proliferation that can result in atherosclerosis (Hu et al., 2012) as well as cardiomyopathy (Lee et al., 2012b). During elevated glucose exposure, autophagy also can impair endothelial progenitor cells, lead to mitochondrial oxidative stress (Martino et al., 2012), and block angiogenesis (Kim et al., 2014).

**mTOR and Stem Cell Proliferation**

In multiple systems of the body, mTOR can influence the proliferation and differentiation of stem cells (Figure 2) (Maiese, 2015)). Stem cell strategies are an important component for maintaining glucose homeostasis during DM (Balestrieri et al., 2013). Loss of the C-terminal six amino acids of mTOR that results in the inhibition of kinase activity decreases embryonic stem cell proliferation (Murakami et al., 2004). Reduced trophoblast growth, faulty implantation, and inability to establish embryonic stem cells occur during the deletion of the mTOR gene (Gangloff et al., 2004). Loss of mTOR activity also leads to cell pluripotency, cell proliferation, and inhibition of mesoderm and endoderm activities in embryonic stem cells (Zhou et al., 2009). Once active, mTOR can foster mesenchymal stem cell senescence (Zhang et al., 2015a). Activation of mTOR also results in stem cell differentiation. For example, increased mTOR and p70S6K activity leads to embryonic stem cell differentiation (Easley et al., 2010).

In the regulation of stem cell proliferation, mTOR is closely linked with the activity of SIRT1 (Zhou et al., 2009) (Figure 2). As noted, SIRT1 inhibits mTOR pathways through AMPK. SIRT1 protects embryonic stem cells during oxidative stress through the induction of autophagy (Ou et al., 2014). SIRT1 blocks mTOR signaling to promote neuronal growth as well as mesangial cell proliferation during high glucose exposure (Zhang et al., 2012). SIRT1 activity is necessary for telomere elongation and genomic stability of induced pluripotent stem cells (De Bonis et al., 2014). SIRT1 can regulate autophagic flux to promote the transition of muscle stem cells from a quiescence state to an active state (Tang and Rando, 2014) and prevent the death of endothelial progenitor cells through autophagy and inhibition of mTOR (Jin et al., 2014). SIRT1 fosters endothelial progenitor cell mobilization and vascular repair during DM in mice (Albiero et al., 2014) and can preserve angiogenesis with bone marrow-derived early outgrowth cells in models of DM (Albiero et al., 2014). In endothelial progenitor cells, SIRT1 prevents cell senescence and impaired cellular differentiation (Lemarie et al., 2011). SIRT1 is required for the angiogenic properties of human mesenchymal stem cells (Chiara et al., 2014). Activation of SIRT1 for stem cell growth may be vital during DM, since patients with Type 2 DM have a down-regulation of endothelial progenitor cells associated with decreased SIRT1 protein levels (Balestrieri et al., 2013). SIRT1 also may function in combination with growth factors to foster improved cardiac performance during glucose depletion through the activation of aged mesenchymal stem cells (Choudhery et al., 2012). In addition, SIRT1 can increase the astrocytic subpopulation of cells that are necessary to support neuronal cell populations (Aranha et al., 2011). SIRT1 may offer stem cell protection through the oversight of mitochondrial pathways. Mitochondrial impairment can occur in endothelial progenitor cells during elevated glucose exposure (Kim et al., 2014). SIRT1 can maintain mitochondrial function during cell injury and block mitochondrial depolarization, cytochrome c release, Bad, and caspase activation (Hou et al., 2010b; Ou et al., 2014).

However, a fine balance in the activities of mTOR and SIRT1 may be required to achieve optimal stem cell survival, proliferation, and differentiation. A decrease in SIRT1 activity that would mirror an increase in mTOR activity is associated with neural differentiation and the maturation of embryonic cortical neurons (Liu et al., 2014a). Differentiation of human embryonic stem cells into motoneurons also occurs with decreased SIRT1 activity. Increased activity of SIRT1 through microRNA-34a also can promote the apoptotic cell death of mesenchymal stem cells (Zhang et al., 2015b).

**mTOR and Metabolic Regulation**

mTOR activation can positively influence cellular metabolism and insulin signaling. Activation of mTOR pathways that involve p70S6K and 4EBP1 can improve insulin secretion in pancreatic β-cells and increase resistance to β-cell streptozotocin toxicity and obesity in mice (Hamada et al., 2009). Loss of p70S6K activity leads to hypoinsulinemia, insulin insensitivity to glucose secretion, glucose intolerance, and decreased pancreatic β-cell size (Pende et al., 2000). Rapamycin administration leads to reduced β-cell function and mass, insulin resistance, decreased insulin secretion, and the onset of DM (Fraenkel et al., 2008). Although inhibition of mTOR activity with rapamycin can limit food intake and prevent fat-diet induced obesity in mice (Deblon et al., 2012), rapamycin can impair glucose uptake and increase mortality in models of Type 2 DM (Sataranatarajan et al., 2015). Rapamycin prevents insulin generated Akt activation and alters the translocation of glucose transporters to the plasma membrane in skeletal muscle (Deblon et al., 2012). Activation of mTOR can protect pancreatic β-cells against cholesterol-induced apoptosis (Zhou et al., 2015) and glucolipotoxicity (Miao et al., 2013). mTOR activation limits vascular disease with atherosclerosis (Peng et al., 2014).

Interestingly, mTOR functions through growth factors to also offer cellular protection during DM (Figure 2). mTOR in conjunction with PI 3-K and Akt can inhibit neuronal cell apoptosis through the epidermal growth factor (EGF) receptor during DM (Kimura et al., 2013). EGF and fibroblast growth factor (FGF) rely upon mTOR to maintain the proliferation of neural stem and progenitor cells (Sato et al., 2010). Erythropoietin (EPO) also is of interest for DM since this trophic factor relies upon mTOR signaling pathways
WISP1 can modulate cellular senescence (Du et al., 2014) to a degree that does not promote excessive cellular proliferation in aging vascular cells (Marchand et al., 2011) that could lead to atherosclerosis during DM. WISP1 also is one of several genes that are over-expressed during pancreatic regeneration, indicating that WISP1 may assist with protection of tissues necessary for metabolic homeostasis (Lim et al., 2002).

WISP1 leads to mTOR activation to block PRAS40 (Shang et al., 2012) and TSC2 (Shang et al., 2013) for cellular protection during oxidative stress. WISP1 regulates the post-translational phosphorylation of AMPK for glucose homeostasis (Chong et al., 2010; Maiese, 2013c; Kopp et al., 2014; Martínez de Morentin et al., 2014). The ability of WISP1 to control AMPK activity is critical to control cellular metabolism during DM (Martínez de Morentin et al., 2014). WISP1 modulates AMPK activation by differentially decreasing phosphorylation of TSC2 at serine1387, a target of AMPK, and increasing phosphorylation of TSC2 at threonine1462, a target of Akt (Shang et al., 2013). This enables WISP1 to provide a minimal level of TSC2 and AMPK activity to control both cell survival and cell metabolism. The level of AMPK activity can become an important factor for cellular survival. Increased AMPK activity can reduce insulin resistance and oxidative stress mediated through the activation of autophagy (Liu et al., 2014b). AMPK activation can correct metabolic parameters of cells and prevent adipocyte differentiation, lipid accumulation, and obesity (Lai et al., 2012). However, under some conditions, AMPK activation promotes apoptotic cell death in pancreatic islet cells in experimental models of Type 2 DM (Guan et al., 2014).

Similar to the importance of controlling the degree of activity for AMPK, controlled activity of mTOR also may prove to provide a vital clinical benefit. mTOR can function in a negative feedback loop and produce glucose intolerance by inhibiting the insulin receptor substrate 1 (IRS-1). In studies with high fat fed obese rats, mTOR leads to inhibitory phosphorylation of IRS-1, impaired Akt signaling, and insulin resistance (Khamzina et al., 2005). Activation of mTOR signaling with p70S6K can phosphorylate IRS-1 in the renin-angiotensin-aldosterone system during consumption of high fat diets that results in high circulating angiotensin II (ANG II) and insulin resistance (Kim et al., 2012).

SIRT1 may be a crucial counterpart to regulate the activity of mTOR during DM. Genes with the greatest statistical change following caloric restriction in mice have included those associated with sirtuin activation and mTOR inhibition (Estep et al., 2009). SIRT1 can increase lifespan in higher organisms (Balan et al., 2008; D’Onofrio et al., 2015; Ma et al., 2015; Maiese, 2015b; Poulose and Raju, 2015), modulates stem cell survival (Hua, 2015; Maiese, 2015a, d; Zhang et al., 2015a, b; Zhang et al., 2015b; Okada et al., 2016), and offers protection against oxidative stress (Hung et al., 2015; Maiese, 2015a, d; Zhang et al., 2015b; Zhang et al., 2015c). Hepatic SIRT1 deficiency results in hepatic glucose overproduction, hyperglycemia, oxidative stress, and inhibition of the gene encoding Rictor that results in impaired mTORC2 and Akt signaling (Wang et al., 2011a), suggesting that SIRT1 is necessary to effectively control mTOR activity. During DM, SIRT1 also can function as a negative regulator of unfolded protein response signaling and inhibit mTOR to lessen hepatic steatosis, insulin resistance,
and glucose insensitivity (Li et al., 2011). In addition, SIRT1 uses AMPK for the regulation of insulin sensitivity. Endothelial cell protection from oxidized low-density lipoproteins requires SIRT1 as well as AMPK activation (Lai et al., 2012; Jin et al., 2014). SIRT1 activation with AMPK may be necessary to protect against spatial memory impairment in combined experimental models of DM and AD. Loss of SIRT1 and AMPK activities can lead to cognitive loss, oxidative stress, and neuronal cell apoptosis (Du et al., 2015).

Conclusions and Future Perspectives
Accompanied by the increase in life expectancy of the global population, NCDs are affecting a greater percentage of individuals in the world. Of particular significance for NCDs is the increasing incidence of DM that now affects at least 350 million individuals. Additional concerns exist for the millions of individuals who are currently undiagnosed and for those individuals that pose high risk for the development of DM, such as those with obesity and impaired glucose tolerance. Current therapies for the management and resolution of DM are limited and lead to significant healthcare costs for the global economy.

New therapies for DM are desperately needed. mTOR is a promising target for launching unique strategies against DM (Table 1). mTOR is an essential component of the protein complexes mTORC1 and mTORC2 and interfaces with PI 3-K, Akt, AMPK, and SIRT1 signaling to affect multiple metabolic parameters that include insulin resistance, insulin secretion, and pancreatic β-cell function. mTOR also plays a significant role in controlling cellular survival during DM.

mTOR offers exciting prospects for the treatment of DM and the multi-systemic complications that can arise from this disorder (Table 1). Yet, several considerations need to be addressed when targeting mTOR for clinical disorders involving cellular metabolism. Under a number of conditions, mTOR may not function independently but works through a PI 3-K, Akt, and mTOR axis. The degree of activity of each of these pathways should be considered that function alongside of mTOR when designing new therapies for DM. mTOR also can exert control over the downstream signaling pathways of AMPK, SIRT1, and WISP1. PI 3-K and Akt are vital in regulating the activity of mTOR and function in tandem with mTOR through growth factor signaling that includes EGF, FGF, and EPO.

As a result, targeting mTOR for clinical benefit must also take account of the dependency mTOR has with PI 3-K and Akt and subsequent downstream pathways. Studies have shown that down-regulation of mTOR and mTORC1 signaling can foster a significant feedback activation of PI 3-K, Akt, and Ras-mitogen activated protein kinase signaling that can counteract any expected clinical benefits (Mäiese, 2015). In addition, inhibition of mTOR signaling can impair glucose uptake, increase mortality in models of Type 2 DM (Sataranatarajan et al., 2015), prevent insulin generated Akt activation, and alter the translocation of glucose transporters to the plasma membrane in skeletal muscle (Deblon et al., 2012). However, increased activity of PI 3-K and Akt signaling may significantly enhance mTOR activity that sometimes limits protective pathways. During such circumstances, drug efficacy, such as with metformin, could be affected. Similarly, long-term mTOR activity may lead to vasculopathy (Sinha et al., 2008).

Levels of mTOR activity also can impact stem cell proliferation and maintenance, such that mTOR activation may be necessary for stem cell proliferation and differentiation. mTOR also functions in combination with Akt to prevent mesenchymal stem cell aging (Zhang et al., 2015a). However, in relation to SIRT1 and stem cell maintenance, a repressed level of mTOR may be more suited to stem cell survival to regulate autophagy pathways and promote stem cell proliferation and differentiation during DM.

mTOR is vital in the regulation of programmed cell death that appears to require a fine balance in the regulation of apoptotic and autophagic pathways. Activation of mTOR is protective against apoptotic cellular injury. During mTOR activation, apoptotic pathways can be averted. mTOR activation during metabolic impairment can protect pancreatic β-cells from cholesterol mediated apoptotic cell injury (Zhou et al., 2015), block the development of vascular complications, such as atherosclerosis (Peng et al., 2014), foster pancreatic β-cell proliferation, and prevent neuronal cell injury (Mäiese, 2015). PI 3-K, Akt, and mTOR can block neuronal cell apoptosis through the EGF receptor during DM (Kimura et al., 2013). EPO also relies upon PI 3-K and Akt with mTOR to protect hippocampus-derived neuronal cells (Ryou et al., 2015) and block endothelial cell injury in experimental models of DM (Chong et al., 2007, 2011). In addition, Akt is vital in maintaining the activity of mTOR through the control of the TSC1/TSC2 complex (Mäiese et al., 2013c).

Yet, autophagy that occurs during mTOR inhibition can have a dual role that either protects cell survival and normalizes metabolic parameters or promotes impairment of some progenitor cells and fosters oxidative stress injury. A reduction in mTOR activity to allow for the induction of autophagy may be necessary for treatment strategies in DM. Loss of autophagy pathways can lead to increased insulin resistance with elevated lipids and inflammation (Lim et al., 2014). Autophagy also may be necessary to control apoptosis. Induction of autophagy can protect cardiomyocytes from apoptotic demise during DM (He et al., 2013). In contrast, autophagy also may promote cellular and tissue pathology during DM. Autophagy may be responsible for the loss of cardiac and liver tissue in diabetic rats during attempts to achieve glycemic control through diet modification (Lee et al., 2014). AGEs in DM can result in the induction of autophagy and vascular smooth muscle proliferation that can result in atherosclerosis (Hu et al., 2012). Induction of autophagy also can injure endothelial progenitor cells and lead to mitochondrial oxidative stress (Mäiese, 2015).

Modulation of mTOR activity to effectively achieve metabolic homeostasis is of great importance. Either independently or in combination with other pathways, regulation of mTOR can be beneficial to treat insulin resistance, limit obesity, promote cellular protection, reduce oxidative stress, and foster tissue regeneration during DM. Further work is necessary to address these challenges to fully comprehend
the impact of the PI 3–K, Akt, and mTOR axis on metabolic homeostasis as well as to elucidate the delicate balance that mTOR and pathways of programmed cell death can exert over cellular survival and cellular metabolism to foster clinical translation of these pathways for optimal clinical success. Given its prominent role in regulating cellular metabolism, mTOR offers a unique target to open new avenues for the development of effect therapies to treat DM and the complications of this disorder.

Table 1 Regenerative Strategies for mTOR in Diabetes Mellitus

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Description</th>
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<tbody>
<tr>
<td>1.</td>
<td>A significant non-communicable disease with limited therapeutic options, diabetes mellitus (DM) is increasing in incidence throughout the world and affects approximately 350 million individuals.</td>
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<td>2.</td>
<td>The mechanistic target of rapamycin (mTOR), a principal component of mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2), offers exciting prospects for the treatment of DM since mTOR is tightly linked to DM cellular metabolism and can involve regeneration and protection of pancreatic β-cells, block neuronal cell apoptosis, protect against cognitive loss, and foster glucose homeostasis.</td>
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<td>3.</td>
<td>mTOR is intimately associated with phosphoinositide 3-kinase (PI 3-K), protein kinase B (Akt), AMP activated protein kinase (AMPK), silent mating type information regulation 2 homolog 1 (Saccharomyces cerevisiae) (SIRT1), and Wnt1 inducible signaling pathway protein 1 (WISP1) to oversee metabolic homeostasis.</td>
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<td>4.</td>
<td>A fine balance for mTOR activation is required to oversee apoptotic and autophagic pathways for cell survival and cell death as well as for control of stem cell proliferation, maintenance, and differentiation.</td>
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<td>5.</td>
<td>Growth factors such as epidermal growth factor, fibroblast growth factor, and erythropoietin rely upon mTOR signaling to modulate insulin resistance, obesity, cellular protection, and oxidative stress during DM.</td>
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References


