The Hippo Signaling Pathway in Pancreatic $\beta$-Cells: Functions and Regulations

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ABSTRACT Hippo signaling is an evolutionarily conserved pathway that critically regulates development and homeostasis of various tissues in response to a wide range of extracellular and intracellular signals. As an emerging important player in many diseases, the Hippo pathway is also involved in the pathophysiology of diabetes on the level of the pancreatic islets. Multiple lines of evidence uncover the importance of Hippo signaling in pancreas development as well as in the regulation of $\beta$-cell survival, proliferation, and regeneration. Hippo therefore represents a potential target for therapeutic agents designed to improve $\beta$-cell function and survival in diabetes. In this review, we summarize recent data on the regulation of the Hippo signaling pathway in the pancreas/pancreatic islets, its functions on $\beta$-cell homeostasis in physiology and pathophysiology, and its contribution toward diabetes progression. The current knowledge related to general mechanisms of action and the possibility of exploiting the Hippo pathway for therapeutic approaches to block $\beta$-cell failure in diabetes is highlighted. (Endocrine Reviews 39: 21 – 35, 2018)

Diabetes, with an estimated number of over 420 million affected people and dramatic increases all over the world, has already gained epidemic character (1). Currently, there are no available therapies that target the cause of diabetes and the loss of functional pancreatic $\beta$-cells. $\beta$-Cell failure (loss of $\beta$-cell function and mass) is a hallmark of both type 1 diabetes (T1D) and type 2 diabetes (T2D) (2–6). The gradual decline in $\beta$-cell function and mass during current therapies highlights the need for improved therapeutic approaches as well as for a better understanding of the molecular changes underlying functional $\beta$-cell loss in diabetes. Programmed cell death or apoptosis is a hallmark of the diminished pancreatic $\beta$-cell mass in both T1D and T2D (2–5, 7–11). It is also important to note that other mechanisms including $\beta$-cell dedifferentiation (12–14) and failure of adaptive expansion due to impaired proliferation (15) have emerged as possible causes for this $\beta$-cell decline in diabetes.

In T1D, the selective autoimmune attack destroys pancreatic $\beta$-cells leading to a lack in insulin production and hyperglycemia. Direct cell-cell interactions and lethal cross-communications between CD$^+$/CD$^+$ T lymphocytes, antigen-presenting cells, and $\beta$-cells, as well as autocrine and paracrine responses through secreted inflammatory cytokines and chemokines, all impact $\beta$-cell death and the development of disease (16, 17). T2D is a complex multifactorial disorder characterized by peripheral insulin resistance and a critical relative decline in $\beta$-cell function and mass. Several diabetogenic stimuli including glucotoxicity, lipotoxicity, islet amyloid polypeptides, and inflammation trigger endoplasmic reticulum and/or oxidative stress leading to impaired insulin secretion and $\beta$-cell apoptosis (18–24).

Pancreatic $\beta$-cell’s insulin secretory response to nutrients, as well as its enormous plasticity to adapt to challenging metabolic demands and cope with such prodiabetic assaults, is governed by the structure and spatiotemporal dynamics of sophisticated signaling networks. Perturbations in signal transductions may occur at different signaling levels, resulting in an imbalance of regulatory networks and subsequent improper activation or repression of genes that could contribute to the regulation of $\beta$-cell mass and function, and thus diabetes progression. The identification of cellular signaling pathways that regulate survival, proliferation, and regeneration of pancreatic $\beta$-cells together with an in-depth knowledge of their mechanisms of action are prerequisites for the comprehensive understanding of disease mechanisms as well as the discovery of new drugs for the treatment of diabetes. The Hippo pathway has emerged as a complex signaling network that controls organ size by regulating cell/tissue homeostasis and behavior; its identification has resulted in an enormous...
ESSENTIAL POINTS

- Hippo pathway regulates pancreas development, including pancreatic progenitor cell proliferation, cell specification and differentiation, and growth and cellular plasticity of the pancreas.
- Hippo pathway components control various aspects of β-cell homeostasis, including β-cell function, survival, and proliferation.
- MST1, a central kinase of Hippo signaling, is activated under diabetic conditions and impairs pancreatic β-cell survival and function.
- MST1 inhibition restores normoglycemia and β-cell function and survival under diabetic conditions in vitro and in vivo.
- The Hippo terminal effector YAP is not expressed in mature endocrine islet cells, and its reconstitution promotes β-cell proliferation and survival.
- Hippo signaling is very complex and dynamic and interacts and cross-talks with many other signaling pathways that regulate β-cell survival, such as the intrinsic apoptotic pathway, mTOR, PI3K-AKT, and MAPK-JNK, to respond to exogenous and endogenous stimuli.
- Targeting Hippo pathway could be a therapeutic approach to restore functional β-cell mass in diabetes.

Hippo Signaling: Basic Knowledge

The Hippo pathway was initially identified through genetic mosaic screens for genes whose loss-of-function mutation leads to tissue overgrowth, increased proliferation, and suppressed apoptosis in Drosophila melanogaster (25, 26, 39–41). Subsequent studies showed that the general components and functions of this pathway are highly conserved in mammals, where it regulates organ size by controlling cell/tissue apoptosis, proliferation, and regeneration (27, 28). The Hippo pathway in mammals comprises the core components mammalian sterile 20-like protein kinase 1 (MST1) and MST2 [homologs of Hpo and also called Serine/threonine-protein kinase 4 (STK4) and STK3], Salvador homolog 1 (Sav1; homolog of Sav), large tumor suppressor 1 (LATS1) and LATS2 (homologs of Wts), MOB kinase activator 1A (MOB1A) and MOB1B (homologs of Mts), the two Yorkie homologs Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), and transcription factor TEA domain family member 1 (TEAD1) through TEAD4 (homologs of Scalloped) (27, 28, 42, 43).

When the Hippo pathway is "on," active MST1/2 phosphorylates Sav1 and MOB1A/B, two adaptor proteins that assist MST1/2 in the recruitment and phosphorylation of LATS1/2 at their hydrophobic motifs (T1079 for LATS1 and T1041 for LATS2) (27, 28, 42, 43). Also MAP4K kinases can phosphorylate and activate LATS1/2, which might explain some MST-independent regulation of LATS and suggests that different inputs into the Hippo signaling could act through distinct kinases (44, 45). Active LATS1/2 subsequently phosphorylates and inactivates transcriptional coactivators YAP and TAZ. LATS1/2 phosphorylates YAP on five (HXRXXS) consensus motifs. YAP S127 phosphorylation promotes 14:3-3 binding, which triggers YAP (and also TAZ) cytoplasmic sequestration and thus inhibition of their transcriptional regulatory activity. Thus, YAP S127 is the major LATS1/2 phosphorylation site, which regulates YAP shuttling. Cytoplasmic YAP and TAZ undergo further phosphorylation leading to eventual ubiquitination-dependent degradation by the proteasomal machinery (42, 46, 47) and thus de-activation of YAP by Hippo (Fig. 1). Conversely, when the Hippo pathway is "off," hypophosphorylated YAP and TAZ translocate into the nucleus and induce their gene expression programs. As transcriptional coactivators, YAP and TAZ are not able to directly bind to DNA. They function to recruit the transcriptional machinery to several transcription factor partners, among the most well-known is the TEAD family, which binds YAP and TAZ and is responsible for the general phenotypes controlled by Hippo signaling (48–50) (Fig. 1). Besides, YAP also interacts with other transcription factors, such as SMADs (51), p73 (52), and RUNX (53).
The Hippo pathway is regulated by a plethora of upstream signals, including cellular stress, cell polarity, mechanical forces, cell-cell interaction, and G protein-coupled receptor (GPCR) signaling (42, 43). Then, a number of transmembrane or cortical proteins, as well as extracellular processes such as cell polarity and cell-cell junction proteins, mechanical cues such as cell contact, GPCR ligands (by regulating Rho Guanosine Triphosphate Pases and actin cytoskeleton reorganization), and the metabolic status such as extracellular glucose and nutrients link an extracellular signal to the cytoplasmic Hippo pathway (42, 43), but it is not completely clear how this works. One key upstream regulator of Hippo signaling is Merlin (also known as Neurofibromin 2 (NF2)), a member of the ezrin/radixin/moesin protein family. Merlin was identified as a tumor suppressor; its mutation causes neurofibromatosis type 2, a benign brain tumor, in humans. Subsequent mechanistic studies in *Drosophila* and mice showed that Merlin directly activates MST1/Sav1 or interacts with LAT51/2 and facilitates LAT51/2 phosphorylation by MST1/2, and thus translates inputs from the extracellular and intracellular cues into the Hippo signaling–regulated gene expression program (54–56) (Fig. 1).

The most recognized functional output of Hippo is to regulate cell survival and proliferation. Human cell cultures as well as *in vivo* mouse models showed that loss-of-function of Hippo elements such as MST1/2, MOB1, LAT51/2, and Merlin, as well as constitutive overexpression of the Hippo terminal effector YAP, leads to increased proliferation and confers a resistance to apoptosis, leading to tissue overgrowth in multiple tissues supporting the functional role of Hippo as a strong regulator of organ size (55, 57–63). Eventually, Hippo pathway dysregulation toward any of its both-opposing ways may lead to severe disease, extensive "off" to cancer with uncontrolled cell overgrowth (28, 42, 64), and extensive "on" to cardiomyopathy and diabetes with specific cell loss (30, 65). Whereas the former has been the subject of extensive investigation in the last decade, the latter is just emerging and is thus the specific focus of this review.

**Hippo Signaling in Pancreas**

In this chapter, we discuss Hippo signaling in the pancreas with the focus on pancreatic islets based on
Hippo in pancreas development

During pancreas development, appropriate temporal and spatial changes in the pattern of gene expression are essential to ensure that each precursor cell in the pancreas differentiates to its required cell fate (66–69). Given the importance of Hippo signaling in regulating cellular behavior, it is perhaps not surprising that Hippo plays an important role in pancreas development, particularly as it plays such a prominent part in the regulation of stem and progenitor cells (70–73). Several independent laboratories discovered that Hippo signaling regulates human and mouse pancreas development and cell-type specification at an early prenatal developmental stage (32–34, 37, 74). The early striking observation was that YAP is initially expressed throughout the developing mouse pancreas, but during cell-type specification, it becomes confined to exocrine cells and is completely lost in endocrine cells following differentiation (32, 33) (Fig. 2). YAP hyperactivation induced by pancreas-specific MST1/2 double knockout in the developing pancreas triggers ductal and acinar cell proliferation and dedifferentiation of acinar cells into duct-like cells that coexpress markers of both cell types (32). These MST1/2 knockout mice have a disorganized, highly immune cell-infiltrated, and smaller pancreas compared with wild-type littermates, which is subject to pancreatitis-like autodigestion (33). Such process is primarily due to a YAP-dependent loss in tissue architecture and integrity in the exocrine compartment and acinar cell dedifferentiation back into ductal cells, as loss of YAP single allele in the MST1/2 knockout mice reversed these structural and functional abnormalities (32). In contrast, compromising the Hippo pathway seems to play a minor role in the endocrine compartment as pancreas-specific MST1/2 knockout mice have relatively smaller islets but show normal glucose homeostasis (33). Of note, transient YAP overexpression in the developing mouse pancreas from E13.5 to E17.5 leads to expansion of ductal cells and impaired differentiation of acinar cells (32). These findings suggest that MST1 and MST2 are critical for maintaining a balanced differentiation status of the exocrine cells but have minimal effects on YAP-depleted endocrine islet cells, proposing a contextual and cell-type-dependent response that occurs during mouse pancreas development (Fig. 2).

A key advance in the understanding of the Hippo pathway in regulation of human pancreas development

Figure 2. Hippo signaling in pancreas development. (a) Although the Hippo pathway is constitutively active to keep YAP activity at low levels in exocrine cells, YAP is not expressed in mature endocrine cells in normal mice. Mice lacking the Hippo core kinase components MST1/2 in the pancreas (PDX1-driven MST1/2 KO) show aberrant YAP expression and activity, which leads to exocrine cell hyperproliferation and dedifferentiation of acinar cells into duct-like cells making acinar-ductal transitional units. Pancreatic endocrine cells of mice lacking MST1/2 have no observable phenotype. (b) Hippo/YAP signaling regulates pancreas development, including pancreatic progenitor cell processes affecting their maintenance, proliferation, and differentiation. YAP levels decrease during pancreas development and inversely correlate with pancreatic differentiation and cell-type specification. Upon transcription factor Ngn3 expression in endocrine progenitor cells, YAP becomes undetectable, which correlates with a low rate in endocrine cell proliferation and regeneration. The onset of Ngn3 expression is sufficient to block YAP expression during endocrine cell differentiation.
came from a recent pioneering study by Cebola et al. (37) using material for human embryonic pancreases. In humans, the YAP/TEAD transcriptional complex binds to and regulates pancreas-specific enhancers activating key pancreatic signaling elements, which regulate proliferation of embryonic pancreatic progenitors during pancreas development. Transcription factor TEAD was the most enriched motif in vitro with binding sites to classically known transcription factors for pancreas development [e.g., forkhead box protein A2 in human embryonic pancreases within 6 weeks after gestation (Caesarean section 16-18) as well as in matching stage of human embryonic stem cell-derived pancreatic multipotent progenitor cells] (37). The YAP-TEAD transcriptional complex acts as a critical enhancer to activate key pancreatic progenitors [e.g., SOX9, pancreatic and duodenal homeobox 1 (PDX1)] during embryogenesis. Active nuclear YAP was also found in the human embryonic pancreas and in human ES cells, as well as in human and mouse pancreatic multipotent progenitor cells and mouse adult pancreatic progenitor cells (37, 74). In contrast, YAP decreases during in vitro pancreatic differentiation and is undetectable also in endocrine progenitor cells, which express transcription factor Neurogenin 3 (Ngn3) (37). Importantly, the onset of Ngn3 expression is sufficient to suppress YAP during endocrine cell differentiation (34).

YAP has never been detected in any of the developing islet cells, in human or rodent adult islets, or in β-cell lines, on messenger RNA (mRNA) or protein levels (32–34, 74). Similarly, TAZ is extremely low but detectable in both adult human and mouse β- and α-cells (75, 76). These changes are important: Loss of YAP is needed to make a β-cell. At a developmental stage, re-expression of YAP in cells, in which YAP is already repressed, blocks differentiation toward endocrine cells. Also, differentiation towards exocrine YAP-expressing cells is blocked by supraphysiological YAP overexpression (32). The opposite strategy, namely silencing YAP, inhibits proliferation of pancreatic progenitor cells in mice (74). The lack of YAP expression correlates with the extremely low rate of β-cell proliferation and β-cell quiescence after birth. Bioinformatics analysis of gene expression of publicly available microarray data identified YAP as a selectively repressed ("disallowed") gene in the pancreatic islet (77). A large-scale follow-up analysis using RNA sequencing datasets of purified mouse endocrine cells confirmed YAP's classification as a "disallowed" gene in islets: it is more repressed in β-cells compared to α-cells (78). This important finding could be the reason for the much lower proliferative capacity of β-cells compared to any other endocrine cell type, including α-cells. Such YAP switch-off would also provide a potential explanation for the strictly regulated β-cell growth to avoid excessive insulin production and hypoglycemia (78).

Fundamental questions are “why” and “how” YAP expression is postnatally muted in endocrine cells. Although little is known, several potential mechanisms can be proposed. Firstly, as suggested in other cell types (79), epigenetic modifications in the YAP gene such as its promoter hypermethylation may be increased in a tissue- and stage-specific pattern, leading to YAP silencing in endocrine cells. Secondly, microRNAs (miRNAs) regulate multiple aspects of β-cell development and function and also control the expression of “disallowed” genes (80, 81). The study by Zhang et al. (74) provides a particularly illuminating example of this miRNA-specific regulation. YAP is a direct target of miRNA-375; its overexpression reduces YAP mRNA expression suppressing proliferation of mouse pancreatic progenitor cells (74). In line with this observation is that miRNA-375 is highly expressed during differentiation of human pancreatic islets together with the induction of insulin transcripts, as well as in zebrafish (82). This potential repression mechanism for YAP has also been reported in the liver, where YAP and miRNA-375 are reciprocally regulated (83). All these studies support the importance of the Hippo pathway in growth and cellular plasticity of the pancreas. The regulation of pancreas development by Hippo/YAP signaling is an evolving topic, and more advances will be required to fully understand this intriguingly intricate process.

Hippo in islet β-cell proliferation and regeneration

As a significant player in tissue homeostasis, Hippo signaling enables cells within a tissue to control their replacement either during physiological turnover such as pregnancy or to induce a healing response following extensive tissue damage. This key aspect of Hippo signal transduction shows its important role in the regeneration of liver, intestine, and heart after damage in mice (84–89). Insights into the control of cell proliferation by the Hippo pathway have also been gained by in vivo mouse studies, which show that overexpression of YAP or inactivation of MST or LATS kinases promote proliferation, leading to the expansion of otherwise quiescent cardiomyocytes or hepatocytes in numbers, which are required to replace dead or damaged cells in liver and heart (86–90).

Pancreatic adult human β-cells are “quiescent” cells with an extremely low rate of replication and limited regenerative capability (91–93), which even further declines with age, suggesting the existence of cell-intrinsic suppressors of growth and mitogenic signaling (94–97). Despite intensive investigations of the underlying principles behind the “quiescence” of β-cells, we still miss the complete picture of the proteins, complexes, and signaling pathways that regulate β-cell proliferation and regeneration, especially in humans, and research has not succeeded to induce robust and controllable human β-cell proliferation for cell therapy. Genetic ablation of MST1
alone (30) or of both MST1 and MST2 (33), which induces proliferation in other cell types, is, under physiological conditions, not sufficient to drive pancreatic β-cells out of quiescence and to induce β-cell proliferation in mice, most likely due to the lack of the Hippo effector YAP. However, we have shown the contextual therapeutic benefit of loss of MST1 in fostering compensatory hyperplasia and restoration of β-cell function in animal models of T1D and T2D (30). Global as well as β-cell specific MST1 deletion in mice preserves β-cell mass by actively enhancing compensatory proliferation of remaining β-cells in the streptozotocin (STZ)-induced β-cell destruction mouse model or in β-cells under metabolic pressure triggered by high-fat diet (HFD) feeding.

To test the hypothesis that re-expression of the Hippo-missing element YAP is sufficient to wake up human β-cells from quiescence, we and the group of Nora Sarvetnick studied the effect of YAP gain of function on adult human islets. George et al. (34) and we (35) independently reported that re-expression of constitutively active YAP (serine 127 to alanine substitution: YAPS127A, which abolishes MST/LATS-mediated YAP inactivation) leads to a robust induction of human β-cell proliferation. YAP-induced proliferation was accompanied by full preservation of the insulin secretory function and β-cell identity genes in the YAP-overexpressing human islets (34, 35). Most of the replicating human β-cells were YAP positive, proposing a mostly cell-autonomous action of YAP. But growth factors secreted from islet cells and/or other uncharacterized secreted proteins induced by YAP re-expression may lead to proliferation in YAP-negative cells by paracrine mechanism. Consistent with this view, connective tissue growth factor (CTGF), an established canonical Hippo/YAP target gene, which is highly upregulated in YAP-reconstituted human islets (34, 35), promotes mouse β-cell proliferation and regeneration in the model of diphtheria toxin-mediated β-cell ablation (98). Consistently, CTGF is essential for mouse β-cell proliferation during embryogenesis, and its transgenic overexpression promotes β-cell proliferation and mass in embryonic insulin-producing cells, proposing that CTGF may act as a proliferative autocrine signal during pancreas development (99). Insights from these preclinical studies will have important implications for the therapeutic targeting of the Hippo pathway and might represent a unique approach to expand β-cells for regenerative therapy of diabetes.

Hippo in islet β-cell survival

Several recent independent findings support the direct regulation of β-cell apoptosis by Hippo pathway components such as Merlin (36), MST1 (30, 31), LAT52 (100, 101), and YAP (35, 38), specifically under increased β-cell stress and metabolic demand in vivo or under diabetic conditions in vitro. The kinase MST1 acts as an essential apoptotic molecule in the presence of diabetic stimuli. MST1 is strongly activated in β-cells in multiple disease models (human and mouse isolated islets, pancreatic sections from autopsy from patients with T2D, β-cell lines) under various diabetic conditions, including proinflammatory cytokines (interleukin-1β (IL-1β) and interferon-γ (IFN-γ)), glucooxygen and glucolipotoxicity, and oxidative stress, suggesting its activation as a common event in the diverse signaling pathways leading to impaired β-cell survival and dysfunction in diabetes (30) (Fig. 3). Importantly, MST1 is activated in isolated islets from diabetic mice, hyperglycemic, obese, HFD mice, and leptin receptor-deficient db/db mice, as well as from patients with T2D; this could be an important mechanism by which MST1 hyperactivity contributes to β-cell pathogenesis (30). In addition to β cells, MST1 is also activated in the kidney of hyperglycemic IRS2-knockout (KO) mice (102), in epididymal fat pads of HFD-treated mice (103), and in rodent cardiomyocytes (104) and podocytes (105) under high-glucose conditions, indicating its disease-associated activation in multiple tissues during diabetes progression. MST1 overexpression induces apoptosis and impaired function in both human and rodent β-cells. Conversely, β-cell deletion of MST1 in mice in vivo or its deficiency in human islets and a rodent β-cell line in vitro protects β-cells from apoptosis and restores β-cell function and normoglycemia (30). Also, RASSF1, an MST1-associated protein, which stimulates the pro-apoptotic function of MST1 (106), is highly upregulated in the pancreas of diabetic mice (107), raising the possibility that this adaptor protein might be able to regulate MST1 actions to induce β-cell death.

Like MST1, overexpression of another Hippo kinase, LAT52, also caused β-cell apoptosis and impaired function (100, 101). Conversely, LAT52 deficiency in the rodent β-cell line INS-1E and in primary isolated human islets (100) as well as LAT52 ablation in mice reduced β-cell apoptosis and ameliorated diabetes development (unpublished data). Correspondingly, Merlin, a critical upstream component of the Hippo pathway, also plays an important role in the regulation of β-cell survival. Loss of Merlin in INS-1E β-cells and isolated human islets leads to the protection from apoptosis triggered by proinflammatory cytokines, as well as glucotoxicity and lipotoxicity (36).

The importance of the Hippo effector YAP for β-cell survival has been highlighted by recent studies. YAP serves as protective signal to confer intrinsic β-cell resistance to apoptosis (35, 38). Re-expression of constitutively active YAP improves human islet and INS-1E β-cells’ survival in response to inflammatory and metabolic stress induced by chronic exposure to cytokines IL-1β and IFN-γ and increased concentrations of glucose and the free fatty acid palmitate, respectively (35, 38). Consistently, all these studies highlight the powerful impact of Hippo signaling manipulations on both human and rodent β-cell survival, suggesting a highly

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regulated Hippo-dependent survival program, which balances β-cell homeostasis.

Mechanisms of Hippo Pathway Dysregulation in Diabetes

Hippo signaling is a complex and dynamic pathway and is fine-tuned by numerous kinases/phosphatases and regulatory adaptor proteins as well as by cross-communication with other pathways to ensure proper responses to exogenous and endogenous stimuli (28, 42, 43). To understand Hippo signaling in diabetes, it is essential to fully dissect the complex processes by which dysregulation of single Hippo components disrupt normal β-cell homeostasis. Multiple mechanisms, which contribute to the dysregulation of Hippo signaling and cause β-cell failure in pancreatic islets in diabetes, are discussed here.

MST1

The functional domains of the kinase MST1 (Box 1) are instrumental in its regulation. Caspases, a family of protease enzymes and key players in apoptosis, play an important role as both effector and regulator of MST1 in the context of cell death. Whereas MST1 serves as an activator of caspases, caspase-mediated cleavage of MST1 elevates its activity, providing the opportunity for a positive feedback loop to amplify the apoptotic signal (122, 125, 129–131). A pathological example of this “feed-forward” mechanism that leads to cell death occurs in primary human and mouse islets as well as INS-1E β-cells under stressful conditions. In this scenario, MST1 activates the caspase machinery in β-cells. Activated caspases cause proteolytic cleavage and further activation of MST1, while caspase inhibition is sufficient to block MST1 cleavage and β-cell apoptosis. Thus, MST1 in the linear signaling sequence receives an additional input from executioner caspases that can profoundly potentiate apoptosis (30, 31) (Fig. 3). This caspase-dependent MST1 cleavage is triggered by multiple types of cellular stress in vitro, such as proinflammatory cytokines, glucolipotoxicity, and oxidative stress, as well as islets isolated from T2D patients and diabetic mice.

MST1 hyperactivation initiates the intrinsic cell death program, which is also induced by multiple diabetogenic stimuli as well as in isolated human islets from patients with diabetes (132). This occurs through specific upregulation of the proapoptotic BCL-2 member Bcl-2-like protein 11 (BIM). Mechanistically, MST1-induced BIM upregulation evokes an increase of the proapoptotic BAX/antiapoptotic BCL-2 ratio, leading to cytochrome c release and subsequent caspase-9 and -3 activation and mitochondrial-mediated apoptosis in the β-cells (Fig. 3). In support of this machinery, the inhibition of the intrinsic apoptotic pathway, through BAX inhibitory peptide or loss of BIM expression, antagonizes MST1-induced β-cell death.

The MST1 signaling output is directly or indirectly regulated by a cross-talk between MST1 and other signaling proteins through complex positive and negative feedback loops to balance cellular homeostasis. Here we highlight three of these regulatory genes that control β-cell survival and function: 1) the serine/threonine c-Jun N-terminal kinase (JNK), 2) the PI3K-AKT pathway, and 3) the transcription factor PDX1.

JNK

Stress-induced MST1 activation and apoptosis is modulated by the cross-talk with kinase JNK (30) (Fig. 3). MST1 activates JNK through the MEKK1-MKK4/JNK1/2 pathway (133) supporting JNK as central mediator of MST1-induced apoptosis (134). However, the concept of such a linear pathway is challenged, as JNK can also act as upstream activator of MST1 through MST1 phosphorylation at Ser82 (116) (Box 1). Thus, the activation of the kinase MST1 following metabolic or inflammatory assaults is part of a general stress response that involves the activation of JNK, as its inhibition blocks MST1 cleavage and intrinsic β-cell death (30). Reciprocally, MST1-overexpressing

Figure 3. Mechanistic model of how Hippo kinase MST1 impairs β-cell survival. Through yet unknown upstream mechanisms, diabetogenic stimuli (i.e., oxidative stress, glucolipotoxicity, and proinflammatory cytokines) lead to the activation of MST1. Activated MST1 upregulates mitochondrial proapoptotic BH3-only protein BIM, which triggers BAX-dependent release of cytochrome c from mitochondria by blocking the antiapoptotic BCL-2 subfamily of proteins BCL-2 and BCL-xL. Cytochrome c binds to APAF1 and gives rise to the apoptosome, which then cleaves and activates initiator caspase-9. Active caspase-9 triggers cleavage of caspase-3, which induces the caspase-3–dependent cleavage of MST1 to its constitutively active fragment leading to further MST1 activation and accelerating β-cell apoptosis by a positive feedback mechanism. The MST1-induced activation of the intrinsic cell death pathway is regulated by JNK and AKT signaling, APAF1, apoptotic protease activating factor 1; BAX, BCL-2-associated X protein; BCL-2, B-cell lymphoma 2; BCL-xL, B-cell lymphoma-extra large; FFA, free fatty acids.

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human islets or INS-1E cells display sustained JNK activation and JNK-dependent apoptosis (30), indicating the existence of a positive feedback loop between these two stress-induced kinases.

**PI3K-AKT**

One of the major signaling pathways that maintain β-cell survival and replication, as well as insulin gene expression and secretion, is PI3K-AKT (134–136). The AKT kinase suppresses apoptosis in a PI3K-dependent manner by phosphorylating, and therefore inhibiting, proapoptotic MST1. MST1 is deactivated by AKT-mediated phosphorylation at its Thr120 and Thr387 residues, which results in inhibition of MST1 cleavage, autophosphorylation, kinase activity, and nuclear translocation (113–115) (Box 1). Reciprocally, both MST1 and its cleaved forms act as direct inhibitors of AKT through direct physical interaction (137). There are several direct indications for the MST1-AKT cross-talk and its bidirectional regulatory mechanism in β-cells. AKT hyperactivation by exogenous mitogen or growth factors or by overexpression of constitutively active AKT1 suppresses MST1 activation and β-cell apoptosis induced by inflammatory cytokines or high glucose. In turn, pharmacological or genetic inhibition of PI3K-AKT signaling promotes MST1 activation and exacerbates stress-induced β-cell apoptosis (30). Thereby, the loss of integrity of β-cell’s AKT signaling in “diabetic” islets (138–140) leads to unrestrained proapoptotic MST1 (Fig. 3).

**PDX1**

In addition to the survival/death signaling pathways described previously, MST1 hyperactivation impairs insulin secretion by direct regulation of PDX1 (30) (Fig. 4). PDX1 regulates many aspects of the β-cell, including cell fate specification, β-cell survival, and expression of insulin and many other genes that are key for glucose sensing and entering to the cell as well as for insulin production (141). We identified PDX1 as a β-cell–specific target of MST1. MST1 physically interacts with and phosphorylates PDX1 at T11, promoting its ubiquitination and subsequent proteasomal degradation, thereby reducing its transcriptional activity and impairing insulin gene expression and secretion (Fig. 4). Such MST1-PDX1 association is stress driven. PDX1 degradation is induced by MST1 hyperactivity under cytokine exposure or high glucose exposure or by its ectopic overexpression (30). Consequently, PDX1-T11 phosphorylation compromises its stability as well as transcriptional activity. PDX1-dependent gene transcription and insulin production is critically impaired in a way that glucose-stimulated insulin secretion is fully abolished. Mutating PDX1-T11

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**BOX 1. MST1 PROTEIN STRUCTURE.**

The secondary structure of MST1 comprises a kinase catalytic domain in the N-terminal, followed by a noncatalytic tail that contains two caspase cleavage sites, two nuclear export signals, and a dimerization domain at the C-terminal. The part of the C-terminal domain (amino acids 431 to 487) is essential for MST1 dimerization (108, 109). Activated MST1 forms homodimers via the Salvador/Rassf/Hippo (SARAH) amino acids 433 to 480 domain at the extreme C-terminal region and heterodimers with SARAH domain of adaptor proteins RASSF and Sav1 (110). In response to apoptotic or stress stimuli, MST1 is autophosphorylated at multiple sites in the activation loop, which results in its activation. Autophosphorylation of Thr183 within the kinase domain is a principal event leading to the activation of MST1 (111, 112). In addition to autophosphorylation, MST1 activity is also regulated by transphosphorylation by several protein kinases such as AKT (at T120 and T387 residues) (113–115), JNK (at S82 residue) (116), c-Abl (at Y433 residue) (117), mTORC2 (at S438 residue) (118), and CK2 (at S320 residue) (119). Although increased phosphorylation levels at S82, T183, and Y433 will lead to MST1 activation, MST1 activity will be inhibited when it is phosphorylated at T120, T387, and S438. There is also evidence showing that activation loop phosphorylation of Thr183 can be mediated by TAO 1/2/3 kinases (120, 121). Two caspase cleavage sites in MST1 (DEMD326↓349 and S351-T370) have been identified (122–124). Importantly, these cleavage sites link catalytic to regulatory domains. Subsequent studies have reported that MST1 kinase activity is highly elevated upon cleavage and release of the 36- to 38-kDa catalytic fragment (125–128). This cleavage also removes the N-terminal catalytic domain from nuclear export signals located in the C-terminal domain, thus promoting nuclear translocation of the kinase domain (129). Genetic disruption of these cleavage sites reduces MST1 kinase activity, its nuclear entry, and its ability to trigger apoptosis (124, 125).

A schematic representation depicting the multiple domains of MST1 and the residues targeted by phosphorylation is shown at the top of the page.
to alanine blocks MST1-induced PDX1 ubiquitination and degradation, allowing PDX1 stability even in the presence of hyperactivated MST1 together with maintaining PDX1 transcriptional activity, insulin transcription, and β-cell function (30). Notably, MST1 deficiency in β-cells in vitro and in vivo restores PDX1 levels in cytokine- and high glucose–treated INS-1E β-cells as well as in the multiple low-dose STZ diabetic mouse model (30). Thus, the MST1-dependent decline in β-cell survival is paralleled with a specific loss of function.

Merlin

Merlin transduces diverse upstream signals to the central core kinases to control the functional output of the Hippo pathway. In a classical model of Hippo initiation, Merlin directly activates the MST/Sav complex that leads to subsequent LATS1/2 activation, proposing the linear cascade of Merlin-MST-LATS (53). However, in the newly suggested intricate “parallel” model, Merlin and Sav1 act as scaffolds to recruit LATS and MST to the membrane. By forming a signaling compartment at the plasma membrane, MST phosphorylates and activates LATS (54). In primary human islets and INS-1E β-cells, loss of Merlin antagonizes high glucose–induced LATS1/2 but not MST1/2 phosphorylation, suggesting the operation of the “parallel” model by which Merlin might directly bind and regulate LATS1/2 phosphorylation in pancreatic β-cells (36, 142). In support of this model, LATS2 reconstitution reverses the protective effect of Merlin deficiency on β-cell survival under glucotoxic conditions (36).

A more thorough understanding of the signals and mechanisms underlying the Merlin-dependent Hippo pathway initiation will be essential to identify functional control of downstream components in the β-cell under physiological and pathophysiological conditions.

YAP

The recent unique and important finding is that reexpressing YAP, which is lost during β-cell maturation, has a pivotal role on β-cell turnover through two mechanisms: firstly, by driving the replication of β-cells and, secondly, by supporting stress adaptation and antiapoptotic response of β-cells under diabetogenic conditions (34, 35). The specific mechanisms as to how YAP promotes human β-cell proliferation are currently being investigated. YAP re-expression in isolated human islets is accompanied by upregulation of its transcriptional partner TEAD, hyperphosphorylation, and inactivation of retinoblastoma protein, as well as induction of a number of cell cycle activators such as myc and cyclin D1. This indicates that YAP directly or indirectly stimulates expression of these cell cycle regulators (34). Another pathway for how YAP overexpression increases β-cell replication in human islets is via the activation of mTORC1. In support of this, YAP overexpression robustly activates mTORC1 in human islets, and

Figure 4. Mechanistic model of how Hippo kinase MST1 impairs β-cell function. In unstressed β-cells, transcription factor PDX1 is located in the nucleus and controls the expression of several genes that are important for glucose sensing and insulin production. PDX1 directly binds to their promoters and induces the expression of genes encoding GLUT2 (SLC2A2), GCK (GCK), and Insulin (Ins). In stressed β-cells, MST1 and its cleaved fragment (which translocates to the nucleus) interact with and directly phosphorylate PDX1 at Thr-11 residue. Phosphorylated PDX1 is marked for ubiquitination and subsequent degradation by the proteasome machinery. This leads to downregulation in the transcription of PDX1-target genes such as SLC2A2, GCK, and Ins, which ultimately impairs insulin secretion. GCK, glucokinase; GLUT2, glucose transporter 2; p, phosphate; Thr, Threonine.
YAP-induced human β-cell proliferation is partly reversed by the mTOR inhibitor rapamycin (34). A third mechanism of YAP-induced β-cell replication involves transcription factor FOXM1. We have shown that overexpression of YAP induces FOXM1 at mRNA and protein levels in human islets, and its inhibition fully blocks human β-cell proliferation, suggesting that upregulated FOXM1 is necessary for YAP-induced β-cell proliferation (35). FOXM1 is a bona fide positive regulator of β-cell proliferation. Pancreas-specific ablation of FOXM1 results in diabetes due to a progressive decline in β-cell mass (143). FOXM1 overexpression promotes β-cell replication in mouse and human islets (144) and is required for the adaptive β-cell proliferation and β-cell mass expansion under demanding metabolic conditions such as pregnancy or insulin resistance (140, 145, 146). Indeed, it has been shown experimentally that the FOXM1 promoter has TEAD binding sites and the YAP/TEAD complex directly binds to the FOXM1 promoter and induces gene expression of FOXM1 and subsets of other proliferative genes encoding positive cell cycle regulators (147). Despite the accumulating mechanistic evidence for the link between mTOR and YAP (148, 149) as well as FOXM1 and YAP (147), these links in the context of β-cell proliferation are still only correlative and require further mechanistic investigations.

Regarding YAP’s efficacy to improve β-cell viability, a distinct redox mechanism has been proposed (35). YAP has been linked to antioxidative stress response under various pathological processes; it controls the expression of several antioxidant enzymes and proteins that detoxify reactive oxygen species (ROS) (65, 150, 151) (e.g., it activates transcription of catalase and superoxide dismutase genes). Subsequently, oxidative stress and ischemia/reperfusion-induced injury in the heart are reduced (65). In human islets and INS-1E β-cells, YAP induces small redox antioxidant proteins thioredoxin-1 (Trx1) and Trx2 that were identified in a screening of ROS-related genes and are necessary for YAP-dependent β-cell protection (35). As expected for balanced cellular systems, the relationship between oxidative stress and YAP is complex and bidirectional: YAP promotes antioxidative responses under normal and pro-oxidant conditions, and in turn, oxidative stress may also be able to degrade YAP (65, 150, 151). In this regard, strong bursts of ROS production by loss of Trx1/2 or metabolic or inflammatory conditions diminishes YAP levels and β-cell survival in human islets. In contrast, YAP reconstitution highly upregulates Trx1/2 and inhibits β-cell apoptosis (35). We speculate that YAP can potentially provide feedback inhibition of MST1 signaling in a redox-dependent manner, through regulation of its target gene regulation Trx1 (152).

Interestingly, TEAD1, a well-established YAP transcriptional partner, is highly expressed in β-cells. Preliminary data show that mice with β-cell–selective TEAD1 disruption show progressive hyperglycemia and diabetes due to severe loss of insulin secretion, suggesting a uniquely important role of TEAD1 in the regulation of β-cell function (153). In the liver, YAP was identified as a strong negative regulator of peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1-α) function, a key regulator of gluconeogenesis. Via suppression of PGC1-α, YAP blocks expression of genes involved in gluconeogenesis in mice. Consistently, liver-specific overexpression of YAP is sufficient to inhibit hepatic gluconeogenic gene expression, lower blood glucose levels, and improve glucose tolerance (154). The existence of such a complex and whether it has any functional roles in β-cells warrant further investigations.

In summary, YAP is absent and not essential for adult β-cell homeostasis, but it is able to turn quiescent cells into proliferative cells and preserves β-cell survival by promoting β-cell protection under diabetic conditions.

**Hippo Signaling as Therapeutic Target**

The important role of the Hippo pathway components as therapeutic target in cancer and other human diseases has been substantiated over the past 10 years (28, 42, 64). The rationale for modulating the Hippo pathway for therapeutic purposes in diabetes has been strengthened by the previously described recent findings indicating that Hippo signaling has important functions in the regulation of β-cell survival and proliferation and insulin secretion. Aberrant MST1 activity is not unique to diabetic β-cells; MST1 hyperactivation contributes to diabetic complications as cardiomyopathy (104, 155), nephropathy (105), and cardiovascular disease (156–158), as well as to neurodegenerative diseases (159–162) and autoimmunity (163). Mice with systemic deletion of MST1 showed promising results in the control of several pathological settings (155, 157–159, 161–163). With the aim of therapeutically exploiting MST1, multiple studies were performed using high-throughput screens to identify potent compounds, which inhibit MST1 activity. The compound gE1 was the first small molecule inhibitor of MST1 identified by screening of an organometallic library (164). gE1 blocks MST1 activity in vitro in a cell culture model. However, one major drawback of this inhibitor is its strong off-target effects on other kinases such as proto-oncogene serine/threonine protein kinase PIM1 (PIM-1) and glycogen synthase kinase 3 (GSK-3β) (164), which precludes its utility for preclinical studies. Other biochemical-based screenings and lead optimization identified LP-945706 (163) as a robust selective MST1 inhibitor. LP-945706 shows promising anti-inflammatory activity and reduces disease progression in an experimental autoimmune encephalomyelitis model (163). By applying an enzyme-linked immunosorbent assay–based, high-throughput biochemical assay, Fan et al. (88) recently discovered the reversible and selective MST1/2
inhibitor XMU-MP-1, which shows good efficacy for MST1/2 inhibition in vitro in several cell types. MST1 blockade by XMU-MP-1 remarkably augments tissue repair and regeneration by promoting cellular proliferation in human liver cells and in a preclinical study using a mouse model of liver and intestine injury (88). In parallel and through another high-throughput screen across a highly privileged collection of 641 drug-like kinase inhibitors together with a triaging strategy, we have recently identified a selective, non-cytotoxic small-molecule MST1 inhibitor (165). This unique inhibitor suppressed MST1 activity and improved β-cell survival in vitro under multiple diabetogenic conditions in human islets and in INS-1E β-cells. In a number of preclinical studies in vivo, the compound restored normoglycemia, β-cell function and survival, and β-cell mass in a type 1 (multiple low-dose STZ) and a type 2 (obese Leprdb/db) diabetic mouse model serving as a potential β-cell protective drug for diabetes therapy (165). It is important to note that the output of MST1 deficiency in terms of survival can vary considerably between different cell types and tissues. Whereas loss of MST1 in liver cells (59), cardiomyocytes (157), astrocytes (162), and pancreatic β-cells (30) protects from stress-induced apoptosis, MST1 deficiency in T-cells (166) results in impaired survival. Thus, targeted strategies that aim for cell-specific MST1 inhibition are a possible solution, although at present, such chemical or biological entities (e.g., cell-specific, ligand-decorated nanocontainers that could deliver their content to a specific cell) are not practically available yet for β-cell-directed therapy.

Taking advantage of YAP’s protective and promoting action involved in both β-cell survival and proliferation, YAP would be another target to induce repair and regeneration of injured and dysfunctional β-cells. However, the lack of YAP expression in adult islets presents an obstacle for this therapeutic strategy, as current MST1/2 inhibitors achieve YAP degradation inhibition and promote its nuclear localization (90) but would not achieve transient re-expression of YAP. Identification of evolutionary mechanisms and the interacting molecules that are responsible for YAP silencing in mature β-cells will enable the development of sophisticated biochemical and molecular strategies that could achieve transient restoration of YAP. Due to the oncogenic potential of YAP, careful design of dosing and timing of YAP induction would be important to ensure that the level of expression achieved is sufficient to induce processes such as β-cell proliferation, while at the same time avoiding chronic activation of YAP that may initiate neoplastic growth.

In summary, targeting Hippo, with its newly discovered inhibitors for MST1 and promising results from preclinical studies, opens a wide range of cell-based therapies to restore β-cell mass, diminish autoimmunity, and minimize diabetes-related complications to be implicated into the future therapy of diabetes.

Open Questions and Future Perspectives

In recent years, the importance of the Hippo pathway in β-cell biology has become increasingly apparent; Hippo signaling regulates key cellular processes in the β-cell such as survival, function, and proliferation. Also, the discovery of Hippo functions in regulating pancreatic progenitor proliferation and cell specification and differentiation during pancreas development has provided missing puzzle pieces. The loss of β-cell’s YAP expression links β-cell identity and differentiation to the high sensitivity to apoptosis together with the β-cell’s extremely low proliferative capacity up to quiescence. Clearly, Hippo signaling can influence cell fate and cell identity (167, 168), but detailed molecular mechanisms of the Hippo pathway in β-cell differentiation remain unclear. Proteomic, gene expression and signalomic analyses will provide important data in this direction in the future. Given the remarkable role of this pathway in cell proliferation and apoptosis, the regulation of the Hippo pathway by multiple signals is not surprising. Yet another necessary research avenue will be to identify the intersection of Hippo signaling with other pathways, as multiple changes in β-cell function, proliferation, metabolic adaptation, stress response, and survival are clearly the consequence of a complex integrative signaling network. For instance, several diabetes-related pathways, which have been identified as major regulators of β-cell function and survival, are reported to cross-talk with Hippo signaling in other cell types (30, 149, 169–171). Despite the recent major advances in the Hippo field described in this review, our understanding of how the Hippo pathway is regulated at normal physiological and

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**BOX 2. OUTSTANDING QUESTIONS**

- How do the temporal and spatial organization and regulation of the Hippo pathway shape pancreas development?
- What are the upstream inputs and molecular mechanisms regulating Hippo activities under physiological and pathophysiological conditions at a diabetic state?
- Why and how is YAP expression postnatally muted in endocrine cells? Does transient YAP re-expression in adult mice enhance functional β-cell proliferation, regeneration, and survival at basal state or under conditions of increased β-cell stress and metabolic demand in vivo? What can stimulate YAP expression in β-cells in vivo and could such strategy be used for β-cell therapy?
- How does Hippo signaling cross-talk and interact with other important β-cell survival pathways, such as mTOR, PI3K-AKT, and WNT signaling?
- How is the Hippo pathway regulated in other cells/tissues that regulate glucose homeostasis?
- What are unique compounds that modulate the Hippo pathway, and can they be therapeutically used for the treatment of diabetes?
pathophysiological conditions at disease state is still at a nascent stage, and there are a number of questions that are important to be answered in the future (Box 2) and that will hopefully help to establish unique and urgently needed tools for a β-cell–directed regenerative therapy for diabetes.

References


REVIEW


