Role of FoxO Proteins in Pancreatic β Cells

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Abstract. Forkhead transcription factors of the FoxO family have important roles in cellular proliferation, apoptosis, differentiation and stress resistance. FoxO proteins also play important roles in metabolism of complex organisms. FoxO1 regulates glucose and lipid metabolism in liver, as well as preadipocyte, myoblast and vascular endothelial cell differentiation. In the hypothalamus, FoxO controls food intake. In this chapter, we review the role of FoxO in pancreatic β cells. Pancreatic β cells secrete insulin to maintain the plasma glucose levels in a strict physiological range. Defects of β cell function cause diabetes. The expression pattern of FoxO1 during pancreatic organogenesis is similar to that of Pdx1, Nkx2.2 and Pax4, transcription factors known to be critical for β cell development. FoxO1 is expressed in a subset of pancreatic duct cells, in which insulin and/or Pdx1 are occasionally expressed. FoxO1 inhibits β cell proliferation through suppression of Pdx1 by competing with FoxA2 and protects against β cell failure induced by oxidative stress through NeuroD and MafA induction. Thus, a series of FoxO1 studies in pancreas suggested that FoxO1 plays important roles in pancreatic β cell differentiation, neogenesis, proliferation and stress resistance. Genetic or pharmacological manipulation of FoxO can be used to prevent β cell failure or aid in the differentiation of uncommitted endocrine progenitors into β cells for transplantation.

Key words: FoxO1, Transcription factor, Diabetes, Pancreas, β cell

FoxO proteins as the orthologs of C. elegans Daf16

The FoxO (Forkhead box-containing protein, O sub-family) family of transcription factors is the closest mammalian homolog of Daf-16, a Caenorhabditis elegans gene product found to be a mediator of extended longevity in this roundworm. In C. elegans, disruption of the function of genes coding for the insulin receptor homolog (Daf-2), PI3-kinase (Age-1) and/or PKB (Akt-1/2) results in dauer formation, which represents growth arrest and concomitant extended lifespan. Mutations of Daf-16 prevent these phenotypes in C. elegans, indicating that FoxO (Daf-16) is a downstream target of insulin receptor (Daf-2)-PI3-kinase (Age-1)-PKB (Akt-1/2) pathway [1, 2]. Mammalian cells express three FoxO isoforms, FoxO1 (FKHR), FoxO3a (FKHRL1) and FoxO4 (AFX) [3]. FoxO1 is the most abundant isoform in liver, adipose tissue, and pancreatic β cells, while FoxO3 and FoxO4 are abundant in kidney and muscle, respectively [4, 5].

Regulation of FoxO function

Conventionally, it has been considered that FoxO proteins are phosphorylated by protein kinase B (PKB) at three residues (corresponding to Thr24, Ser253 and Ser316 in mouse FoxO1), resulting in export of FoxO from the nucleus to the cytoplasm [6–12]. Insulin and growth factors negatively regulate FoxO transcriptional activity through phosphorylation and nuclear exclusion induced by PI3-kinase/PKB pathway. On the other hand, recent studies have revealed that other kinases phosphorylate and regulate FoxO. The c-Jun-N-terminal kinase (JNK) phosphorylates Thr447 and Thr451 residues in human FoxO4, which leads FoxO4...
nuclear translocation [9, 13]. The inhibitor of nuclear factor KB kinase (IKK) phosphorylates Ser644 residue in human FoxO3a, which results in ubiquitin-dependent degradation of FoxO3a [14]. Lehtinen et al. reported that mammalian Ste20-like kinase 1 (MST1) phosphorylates FoxO3a, leading nuclear translocation of FoxO3a [15]. Also Huang et al. showed that FoxO1 is phosphorylated by the cycline-dependent kinase 2 (CDK2), which leads nuclear exclusion of FoxO1 [16]. Furthermore, the posttranslational modifications other than phosphorylation have been also reported to regulate FoxO activity. Upon oxidative stress, the cAMP response element binding protein (CREB) binding protein CBP and p300 acetylate FoxO proteins, while the NAD-dependent Sirtuin deacetylates FoxO [17–21]. Although controversial, most authors agree that acetylation targets FoxO to the nucleus and prevents ubiquitin-dependent degradation, while deacetylation increases transcriptional activity of FoxO. Thus, acetylation/deacetylation of FoxO proteins appear to be important for their functions against oxidative stress.

Role of FoxO at the cellular level

FoxO proteins inhibit cellular proliferation through induction of cell cycle inhibitors, such as p27kip1 [22] and p21waf1 [5]. FoxO also promotes apoptosis [7, 23] and premature senescence [21, 24]. Exposure to oxidative stress during the S phase of the cell cycle results in apoptosis [25], while exposure to oxidative stress in the G1 or G2/M phase induces premature senescence [26]. FoxO may contribute to these cellular responses against oxidative stress. FoxO also enhances resistance to oxidative stress through induction of antioxidant enzymes, catalase and Mn-superoxide dismutase (Mn-SOD) [27, 28]. Thus, FoxO proteins play important roles to prevent somatic mutations induced by DNA damage upon oxidative stress.

Signaling cross-talk mediated by FoxO

FoxO is primarily involved in the insulin/IGF-1 signaling pathway as a downstream target of PKB. Insulin/IGF-1 signaling pathway integrates with other intracellular signaling pathways and plays a crucial role in the adaptation of living cells to a wide variety of external stressors. Transforming growth factor-β (TGF-β) signaling interacts with FoxO through direct binding of TGF-β target smad3/smadd4 complex to FoxO proteins [29]. IκB kinase (IKK), a regulator of IκB/NF-κB pathway, phosphorylates FoxO3a as well as IκB, and controls protein stability of FoxO3a [14]. β-Catenin, a multifunctional protein that mediates Wnt signaling, interacts with FoxO and enhances its transcriptional activity [30]. FoxO inhibits leptin signaling in hypothalamus through competition with the signal transducer and activator transcription-3 (STAT3) for binding to the promoter of neuropeptide genes [31]. Furthermore, FoxO interacts with Notch signaling through direct binding to Notch target RBP-Jκ (also called as Csl, CBF-1 or SuH) (Kitamura et al. submitted). This evidence indicates not only a variety of FoxO’s cellular functions but also important mechanisms by which growth factor (insulin/IGF-1) signaling integrates with other signaling pathways.

Role of FoxO in metabolism (liver, adipose tissue, muscle, blood vessel and hypothalamus)

In liver, FoxO1 regulates glucose production through transcription of gluconeogenic genes, G6Pase and PEPCK [32–35]. FoxO1 suppresses expression of genes involved in glycolysis and lipogenesis, including glucokinase and SREBP-1c [36]. Insulin suppresses hepatic glucose production by inhibiting FoxO-dependent transcription. FoxO also regulates the transcription of apolipoprotein CIII (apo CIII), which probably contributes to hypertriglyceridemia in the insulin-resistant state [37]. Thus, FoxO1 regulates multiple aspects of glucose and lipid metabolism in the liver. In adipose tissue, FoxO prevents preadipocyte differentiation. Interestingly, heterozygous FoxO1 knockout mice exhibit reduced levels of serum TNFα and resistin, cytokines known to be associated with insulin resistance. Consequently, these mice are protected from diet-induced diabetes [5]. In muscle, FoxO1 prevents myoblast differentiation through the interaction of FoxO1 and Notch signaling [38] (the authors unpublished data). Skeletal muscle specific FoxO1 transgenic mice showed down-regulated type 1 muscle fibers [39]. FoxO1 also induces muscle atrophy through the increase in the atrophy-related ubiquitin ligase atrogin-1 [40]. Furthermore, FoxO stimulates fatty acid uptake and fatty acid utilization in muscle through regulation of the fatty acid transfer protein.
CD36 and lipoprotein lipase (LPL) [41, 42]. Moreover, FoxO4 regulates vascular smooth muscle cell differentiation through interactions with Myocardin [43]. FoxO proteins have also been implicated in vascular endothelial cell differentiation [44]. FoxO1 knockout mice are embryonic lethal due to incomplete vascular development, which also suggests that FoxO1 plays an important role in blood vessel differentiation [45, 46]. By contrast, both FoxO3a and FoxO4 knockout mice are viable and grossly indistinguishable from their littermate controls [45]. This genetic evidence indicates that FoxO1, but not FoxO3 or FoxO4 is indispensable for organ development. In the brain hypothalamus, FoxO1 controls food intake through the regulation of orexigenic (Agrp) and anorexigenic (Pomc) neuropeptides [31, 47].

![Fig. 1.](image)

FoxO1 is exclusively expressed in islet β cells in adult pancreas. Double immunofluorescence was performed with anti-FoxO1 (red) and anti-insulin (green) antibody on the mouse pancreas section. Overlapping images of FoxO1 and insulin staining are shown in the right panel. The complete overlap (yellow) indicates that FoxO1 is exclusively expressed in insulin-immunoreactive β cells.

![Fig. 2.](image)

Fig. 2. Haploinsufficiency for FoxO1 restores β cell mass in Irs2 knockout mice. The panels show glucagon and insulin staining on pancreatic sections in wild-type, Irs2−/−, Irs2−/−Insr+/− and Irs2−/−FoxO1+/− mice. Compared to wild-type mice, β cell area is significantly reduced in Irs2−/− mice with a slight decrease in α cell area. Insulin receptor (Insr) haploinsufficiency enhances the reduction of β cell area in Irs2−/−Insr+/− mice. By contrast, haploinsufficiency for FoxO1 dramatically restores β cell area in Irs2−/−FoxO1+/− mice. Consequently, Insr haploinsufficiency increases the severity and accelerates the onset of diabetes in Irs2−/− mice. Conversely, FoxO1 haploinsufficiency rescues diabetes in Irs2−/− mice (adapted from Kitamura et al., 2002 [4]).
Role of FoxO in pancreatic β cell growth

In contrast to thus far mentioned organisms (liver, adipose tissue, muscle, blood vessel and hypothalamus), relatively fewer efforts have been made to clarify FoxO’s role in the pancreas. It is important to note that in adult pancreas, FoxO1 is exclusively expressed in islet β cells (Fig. 1), but not expressed in acinar cells or the other islet cell types, such as α cells, δ cells and PP cells (the authors’ unpublished observations). Pancreatic β cell secretes insulin to maintain the plasma glucose levels in an appropriate physiological range. Absolute or relative defects of β cell mass and peripheral insulin resistance [50, 51]. Genetic studies revealed that haploinsufficiency for FoxO1 restores β cell mass and consequently rescues diabetes in IRS2−/− mice (Fig. 2). Importantly, haploinsufficiency for FoxO1 restored Pdx1 expression in IRS2−/− mice [4]. The transcription factor Pdx1 plays a crucial role in β cell growth and function [52, 53]. Pdx1 transcription is regulated by another forkhead transcription factor FoxA2 (also known as HNF3β) [54]. FoxO1 and FoxA2 share common DNA binding sites in the Pdx1 promoter. FoxO1 competes with FoxA2 for binding to Pdx1 promoter, which results in inhibition of Pdx1 transcription [4]. Consistent with the hypothesis that FoxO1 is a negative regulator of Pdx1, FoxO1 and Pdx1 show mutually exclusive nuclear localization. In Pdx1-positive β cells, FoxO1 localizes to the cytoplasm, while in Pdx1-negative β cells, FoxO1 localizes to the nucleus [4]. This hypothesis has been also supported by recent studies showing that in β cells exposed to oxidative stress, FoxO1 translocates from the cytoplasm to the nucleus, while Pdx1 translocates from the nucleus to the cytoplasm [55]. Furthermore, it has been reported that the reduction of β cell mass in β cell specific insulin receptor knockout mice (βIRKO) is accompanied by nuclear restriction of FoxO1 [56]. Taken together, FoxO inhibits β cell growth (proliferation) through suppression of Pdx1. Insulin/IGF-1 signaling controls β cell mass via its regulation of FoxO activity.

Role of FoxO in pancreatic β cell differentiation

Pdx1 is expressed broadly in pancreatic epithelium in early stage of embryo (e9.5–e14.5), while as organogenesis proceeds, becomes progressively restricted to endocrine cells (e14.5–e18.5), then is eventually limited to β cells in mature pancreas [57–60]. The expression pattern of FoxO1 during pancreas organogenesis is identical to that of Pdx1, although subcellular localization of these two proteins are consistently opposite, i.e., nuclear Pdx1 and cytoplasmic FoxO1 (the authors’ unpublished data). Since Pdx1 is required for pancreas morphogenesis, FoxO may also play a role in pancreas development. Interestingly, the other transcription factors that are known to regulate islet cell type specification, such as Nkx2.2, Nkx6.1 and Pax4, also exhibit a similar expression pattern during pancreatic organogenesis [61–63]. FoxO may also play an important role in islet cell type specification. Notch signaling regulates developmental lineage of pancreatic endocrine and exocrine cells through induction of Hes1, a transcriptional repressor of Neurogenin3 [64, 65]. Neurogenin3 is an important transcription factor for endocrine cell lineage specification [66, 67]. FoxO1 interacts with Notch signaling to regulate Hes1 gene expression (T.Kitamura et al. submitted). Thus, FoxO may also account for Notch associated endocrine pancreas development.

It has long been controversial whether new β cells arise from preexisting β cells (via replication) or from stem/progenitor cells (via neogenesis or differentiation). Although recent studies strongly support the former mechanism [68, 69], there is considerable interest in the hypothesis that β cells are generated by differentiation from pancreatic duct cells or neogenesis from pancreatic stem cells [70–73]. Indeed, new islets often arise in close to pancreatic ducts. Insulin and/or Pdx1 expressing cells are occasionally observed in pancreatic ducts, although at a low frequency [74]. Many experimental conditions, such as partial pancreatectomy, ductal ligation, cellophane wrapping of the head of pancreas and GLP-1/exendin4 treatment are associated with increased numbers of insulin-positive duct cells [75–78]. FoxO1 and Pdx1 are expressed in a rare subset of pancreatic duct cells [4]. It is noteworthy that the majority of FoxO1-positive duct cells do not express insulin or Pdx1, whereas all the insulin/Pdx1-positive duct cells are FoxO1 positive. It remains to be seen whether the FoxO1-positive/insulin-
Role of FoxO in pancreatic β cell protection against oxidative stress (glucose toxicity)

It has been proposed that the β cell failure commonly seen in type 2 diabetes is caused by chronic exposure to elevated glucose concentration, through a mechanism called "glucose toxicity". When intracellular glucose concentration exceeds the glycolytic capacity of the β cell, glucose is shunted to enolization pathways, leading to superoxide production [79]. Since β cells express low levels of antioxidant enzymes, such as catalase, Mn-superoxide dismutase (Mn-SOD) and glutathione peroxidase, these cells are more sensitive to oxidative stress compared to the other cell types [80, 81]. In most cell types, FoxO shuttles between nucleus and cytoplasm. In contrast, in β cells FoxO1 constitutively localizes to the cytoplasm due to the continuous stimulation by endogenously produced insulin [82]. Indeed, even prolonged depletion of serum and/or glucose from the medium of β cell culture affects neither phosphorylation nor localization of FoxO1 [21]. In contrast, exposure to oxidative stress results in nuclear redistribution of FoxO1 in β cells (Fig. 3) [21]. Nuclear redistribution of FoxO1 is associated with increased expression of NeuroD and MafA. We have shown that both genes are direct FoxO1 targets [21]. Since NeuroD and MafA are insulin gene transcription factors, the role of FoxO redistribution could be to protect against β cell failure induced by oxidative stress (hyperglycemia). Indeed, MafA expression is decreased in the mouse models of diabetes, and this decrease can be prevented by FoxO1 overexpression in β cells [21]. The molecular mechanism by which FoxO1 redistributes to the nucleus in response to oxidative stress has been recently charac-
terized [9, 13, 21, 55]. In response to oxidative stress, FoxO1 is acetylated by the nuclear coactivators Cbp/p300. Acetylated FoxO1 binds to the promyelocytic leukemia-associated protein Pml and is targeted to nuclear subdomains (Pml nuclear bodies). Acetylation also prevents FoxO1 ubiquitination and degradation, leading to FoxO1 nuclear retention. Upon association with Pml bodies, FoxO1 is deacetylated by the NAD-dependent deacetylase (Sirtuin) Sirt1, which also localizes to Pml bodies. FoxO transcriptional activity is increased by deacetylation, providing the molecular underpinning for increased expression of FoxO target genes, such as NeuroD or MafA. However, as the deacetylated form of FoxO is efficiently ubiquitinated, this leads to increased FoxO degradation [21]. Thus, under oxidative stress, FoxO activity is regulated by a delicate balance between acetylation and deacetylation to prevent excessive FoxO activation, which may result in irreversible apoptosis [7] or cellular atrophy [40, 83]. A model of FoxO regulation mechanism under oxidative stress in β cells is shown in Fig. 4.

**Conclusion**

It is hoped that genetic or pharmacological manipulation of FoxO can be used to treat β cell failure in diabetic patients or for differentiation of uncommitted endocrine progenitors into β cells for regeneration therapy of diabetes.

**References**

294–297.


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