Chapter 20
Glutathione Peroxidase 1 and Diabetes

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Abstract Discovery of development of type 2 diabetes-like phenotypes in glutathione peroxidase-1 (GPx1) overexpressing mice reveals a novel function of this "oldest" and most abundant selenoprotein in the body. The finding signifies an exciting progress in Se biology, and helps understand metabolic impacts of Se supplementation on human health. While its dual role in coping with reactive oxygen and nitrogen species has received broad recognition, unique functions and mechanisms of GPx1 in β cell physiology, insulin synthesis and secretion, and body glucose homeostasis are just being unveiled. By modulating intracellular redox status, the GPx1 overproduction or knockout is able to regulate functional expressions of key transcriptional factors or protein in pancreatic islet and insulin-responsive tissues.

20.1 Introduction

Six forms of glutathione peroxidase (GPx) enzymes have been found in mammals. Among them, GPx1 [1] is an 84 kDa tetrameric protein that was the first identified and the most abundant Se-dependent enzyme. Mainly located in the cytoplasm, GPx1 is able to catalyze the reduction of H_2O_2 and organic hydroperoxides using GSH as the cofactor [2]. Due to this property, GPx1 has been widely considered to be among the major intracellular antioxidant enzymes in vivo. In fact, physiological importance of GPx1 activity in antioxidant defense was clarified using the GPx1

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knockout mice [3]. Knockout of GPx1 leads to increased susceptibility of liver and lung to toxicities of paraquat and diquat that induce the generation of reactive oxygen species (ROS) [4, 5]. While supplementation of high levels of dietary vitamin E in GPx1 knockout mice did not provide the same protection as in wild-type mice [6], GPx1 overproduction conferred extra protection against acute oxidative stress induced by ROS generators [7]. In contrast, knockout of GPx1 actually protected mouse primary hepatocytes against authentic peroxynitrite toxicity [8, 9] or mice against overdose of drugs such as acetaminophen that induces formation of reactive nitrogen species (RNS) [10]. Apparently, GPx1 exerts a dual role in coping with oxidative stress initiated by RNS vs. ROS [3, 11].

20.2 Association of Glutathione Peroxidase 1 with Diabetes

Although alteration of GPx1 expression is implicated in pathogeneses of several chronic diseases [12–15], its link to diabetes and the clinical significance have attracted serious attention only very recently. Diabetes mellitus is a group of metabolic diseases that will affect more than 10% of the American population in the coming decade [16]. Major types of diabetes include: type 1 diabetes (referred to as insulin-dependent diabetes, IDDM), type 2 diabetes (referred to as non-insulin-dependent diabetes, NIDDM), gestational diabetes, and maturity onset diabetes of the young (MODY). The ultimate pathogenesis of diabetes, regardless of type, is insufficient functional insulin in the circulation to maintain body glucose homeostasis, resulting from either defective insulin production or insulin insensitivity.

20.2.1 Islet Physiology and Free Radical Biology

Pancreatic islets of Langerhans constitute approximately 1–2% of the mass of the pancreas that represents a crucial endocrine structure for regulating body glucose metabolism and homeostasis. There are five main types of cells in islets that produce and secrete various hormones: α cells for glucagon, β cells for insulin and amylin, δ cells for somatostatin, PP cells for pancreatic polypeptide, and ε cells for ghrelin. As a major portion of islets, the β cells are highly efficient in glucose uptake upon exposure to rising glucose supply. Thus, extracellular hyperglycemia readily causes intracellular hyperglycemia in β cells. Consequently, auto-oxidation of glucose in these cells may elevate ROS production [17] and cause upward changes in oxidative stress markers such as 8-hydroxy-2-deoxyguanosine and 4-hydroxy-2,3-nonenal [18]. Intriguingly, pancreatic islet cells produce a relatively low amount of antioxidant enzymes including GPx1, superoxide dismutase (SOD), and catalase. Compared with the liver, islets contain only 1% of catalase, 2% of GPx1, and 29% of SOD1 activities, respectively [19–21]. Thus, β cells are considered to be susceptible to oxidative stress that can be induced by hyperglycemia. This perception was supported by the fact that β cells are a primary target of the diabetogenic agents, streptozotocin and alloxan, that generate ROS including H_2O_2 [22, 23].

20.2.2 Earlier Evidence and Perception on the Link of Antioxidants to Diabetes

The predicated susceptibility of β cells to oxidative injury has given a good reason to link antioxidants to diabetes. In addition, there are at least two more mechanisms for free radicals and antioxidants to be implicated in diabetes and insulin resistance [16, 24–26]. One is the responsiveness to ROS by key regulators of β cells and insulin, such as the transcription factors, pancreatic duodenal homebox 1 (PDX1) and forkhead box A2 (FOXA2), and mitochondrial protein uncoupling protein 2 (UCP2) (see below). The other is the oxidative modification of insulin signal proteins by ROS in insulin target tissues. As activation of serine/theronine in protein kinase B (AKT) leads to the translocation of glucose transporter 4 to the cell membrane for glucose uptake [27], impaired AKT activation is associated with insulin resistance [21]. Acting as an oxidative inhibitor of protein tyrosine phosphatase [28], ROS including H_2O_2 modulate the insulin-induced phosphorylation of the insulin receptor β-subunit [25] and AKT on Ser^{473} [29]. Because protein tyrosine phosphatases function as negative regulators of insulin signaling [28], normal physiological levels of ROS are required for sensitizing insulin signaling [30].

Nevertheless, the common perception, until very recently, was that ROS and RNS were detrimental to β cells or insulin action and that upregulating antioxidant defense in islets or whole body was beneficial to prevent and treat insulin resistance and diabetes [26]. Seemingly, there were circumstantial data from experimental and clinical studies to justify this “prevailing” notion. From the Se biology standpoint, inorganic Se was found to act as an insulin-mimic [31, 32]. Dietary Se deficiency was correlated with abnormal glucose and lipid metabolism [33], whereas decreased plasma Se concentrations or selenoperoxidase activity were detected in diabetic subjects [34]. Likewise, levels of the superoxide-scavenging enzyme extracellular-SOD were shown to be inversely related to fasting plasma glucose, insulin resistance, and incidence of diabetes [35–37]. A functional polymorphism of MnSOD was also associated with the incidence of diabetes [38]. Supplemental antioxidants delayed diabetic nephropathy [39]. A comprehensive analysis of this topic can be found in a recent review [26].

20.2.3 Recent Findings on Pro-Diabetic Roles of GPx1

It was striking for us to find that GPx1 overexpressing mice became obese at 6 months of age in the course of determining their increased resistance to various oxidant exposures [40]. Subsequent characterization indicated that these mice developed hyperglycemia, hyperinsulinemia, hyperlipidemia, and insulin resistance, along with elevated pancreatic β cell mass, islet insulin secretion, plasma leptin concentration, and hepatic lipogenesis [40–42]. In contrast, knockout of GPx1 and SOD1 alone or together resulted in decreases in pancreatic β cell mass, plasma insulin concentration, and glucose-stimulated insulin secretion [42]. But, body insulin

sensitivity was improved in these knockout models. Meanwhile, there was a strongly positive correlation between erythrocyte GPx1 activity and insulin resistance in pregnant women with gestational diabetes [43]. A β cell-specific overexpression of catalase or metallothionein in nonobese diabetic mice accelerated onset of diabetes [44]. High glucose led to increased selenoprotein P mRNA expression and protein secretion in rat hepatocytes [45]. Treating these cells with the anti-hyperglycemic drug metformin produced a dose-depended decrease in selenoprotein P mRNA and protein, suppressed glucocorticoid-stimulated production of selenoprotein P, and downregulated mRNA expression of selenophosphate synthetase 2 (an enzyme essential for selenoprotein biosynthesis). Because selenoprotein P is the major transport form of Se, diminishing Se supply to extrahepatic tissues may be one of the mechanisms for the antidiabetic action of metformin [45].

20.2.4 Clinical Relevance of the Pro-Diabetic Role of GPx1 in Human Health

The scientific significance and clinical implication of the type 2 diabetes-like phenotypes induced by GPx1 overproduction have been recognized after a post-hoc analysis of the Nutrition Prevention Cancer (NPC) trial revealed a more than two-fold increase in type 2 diabetes incidence in the Se supplemented compared to the placebo group [46]. A similar trend was also seen in the prematurely terminated Selenium and Vitamin E Cancer Prevention Trial (SELECT) [47]. Most recently, multivariate logistic regression analyses of the ORDET cohort study [48] in Northern Italy and large cross-sectional analyses within the US Third National Health and Nutritional Examination Survey (NHANES 1988–1994; 2003–2004) revealed a strong positive correlation between Se intake or serum Se concentration and the prevalence of type 2 diabetes [49, 50]. Moreover, high body Se status was associated with adverse plasma lipid profiles in adults of the USA, UK, and Taiwan [51–54], although mixed effects of Se on diabetic risk or blood glucose were shown in two French studies [55–57], two small case-control European studies [58, 59], and the US Health Professionals Follow-up study [60]. While more basic and clinical research will be needed to elucidate the full metabolic spectrum of Se in glucose homeostasis and diabetes, illustrating the pro-diabetic role of GPx1 overproduction in mice provides a plausible mechanism to explain the adverse effects of Se super-nutrition on glucose metabolism in humans.

20.3 Mechanisms of Glutathione Peroxidase 1 on Diabetes

As discussed above, ROS are able to interact with key regulators of islet β cell mass and insulin synthesis, secretion, and sensitivity. Thus, the metabolic phenotypes of the GPx1 overexpression and knockout mice were presumably mediated by the

redox regulation of those key factors, because altering GPx1 expression resulted in detectable changes in intracellular ROS status in islets and presumably other tissues as well [42, 61].

20.3.1 Regulation of Islet β *Cell Mass and Insulin Synthesis*

Maintaining pancreatic islet β cell mass is recognized as a pivotal prevention from pathogenesis of both types 1 and 2 diabetes [62]. Regulation of the islet β cell mass takes place at neogenesis, replication, and survival. Transcriptional factor PDX1 is the best known and probably the most important regulator for β cell differentiation and survival as well as expression of the insulin gene and many other genes related to glucose metabolism [63, 64]. Importantly, expression and function of PDX1 are affected by intracellular ROS via a posttranscriptional defect in PDX1 mRNA splicing [65], nucleo-cytoplasmic translocation of the protein [66], and phosphorylation of Ser^{61} and/or Ser^{66} on the protein [67]. In fact, GPx1 overproduction resulted in an upregulation of PDX1 mRNA and protein in islets, along with an attenuated degradation (phosphorylation) of PDX1 protein. The decrease in phosphorylated PDX1 protein in GPx1 overexpressing mice was likely due to a less oxidative environment in islets, as shown by the lower intracellular ROS levels and attenuated phosphorylation of c-jun terminal kinase (JNK) protein. The reduced phosphorylation of Thr^{308} at AKT could partially account for the decreased phosphorylation of PDX1 protein [67–69]. Consequently, an elevated functional PDX1 protein in islets led to hypertrophy of β cell mass and increased pancreatic and plasma insulin concentrations [63, 70–72]. In contrast, the reverse was induced by the GPx1 knockout [42].

Demonstrating hyperacetylation of histone 3 and 4 (H3 and H4) in the PDX1 gene promoter of the GPx1 overexpressing mice [61] unveiled a novel epigenetic regulation of this key transcriptional factor in vivo. Hyperacetylation of H3 and H4 has been suggested to precede transcriptional activation [73, 74], which may help explain the increased islet PDX1 mRNA levels in the GPx1 overexpressing mice. Seemingly, the overproduced GPx1 activity was able to remodel chromatin at the PDX1 promoter to form a more accessible structure for transcription [75]. This remodeling was likely mediated by modulating intracellular ROS status, because the genotype difference in H3 and H4 acetylation was correlated well with that of intracellular ROS levels [61]. Moreover, GPx1 overproduction protected the PDX1 promoter from the H_2O_2-induced H3 and H4 deacetylation [61].

Another important transcriptional factor for β cell differentiation and survival is NeuroD/Beta2 that was also upregulated by the GPx1 overproduction [41]. However, effects of GPx1 overproduction on islet FOX2 mRNA levels were not statistically significant [41]. In vivo, FOXA2 binds the PDX1 gene promoter/enhancer to activate the gene transcription [76]. It is very intriguing that while both GPx1 and SOD1 knockouts decreased pancreatic PDX1 protein levels, only the SOD1 knockout decreased islet FOXA2 mRNA and protein levels and the binding of FOXA2 protein to the PDX1 promoter [42]. Apparently, the regulation of FOXA2 was more

superoxide-dependent [26]. Unlike the GPx1 overproduction [61] or the SOD1 knockout, the GPx1 knockout did not affect islet PDX1 mRNA and H3 and H4 acetylation [42]. Possibly, the extremely low baseline of GPx1 activity in pancreatic islets precluded a detectable response to the gene knockout.

An increased activation of p53 protein (phosphorylation on Ser^{15}) in islets of the GPx1 knockout mice, similar to that by the SOD1 knockout, might also contribute to their decreased islet β cell mass [42]. In diabetic subjects, the β cell apoptosis seems to be a more deciding factor than replication compared with control subjects [77]. This event can be triggered by high glucose [78] and cytokines that induce ROS and RNS formation [79]. However, it is hard to explain why double knockout of GPx1 and SOD1 did not elevate islet p53 activation [42] and why overproduction of GPx1 actually upregulated islet p53 mRNA [41]. It is also fascinating to notice that the hypertrophy of islet β cell mass and upregulation of insulin production seems to be a unique feature of GPx1 overproduction, because insulin content or insulin gene expression in islets was not altered by overexpressing catalase up to 50-fold [44, 80], two forms of metallothionein up to 30-fold [44, 81], or three forms of SOD enzymes up to tenfold [82, 83].

20.3.2 Regulation of Islet Insulin Secretion

Mitochondrial membrane potential is considered to be a driving force for insulin secretion by β cells [84]. As the only uncoupling protein present in rodent and human β cells, UCP2 negatively regulates mitochondrial membrane potential and inhibits glucose stimulated insulin secretion (GSIS) [85, 86]. Therefore, the accelerated GSIS and hyperinsulinemia in the GPx1 overexpressing mice can be well explained by the downregulated islet UCP2 protein and elevated mitochondrial member potential [61]. Treating islets of wild-type mice with the GPx1 mimic ebselen duplicated suppression of UCP2 protein by GPx1 overproduction. Meanwhile, knockout of GPx1 alone or together with SOD1 upregulated UCP2 protein in pancreas and decreased islet ATP content [42]. Both changes could contribute to the attenuated GSIS in these mice.

20.3.3 Regulation of Insulin Signaling in Insulin Target Tissues

The fact that H_2O_2 serves as a major substrate of GPx1 allows the enzyme to affect insulin sensitivity at multiple sites because H_2O_2 may activate or prolong phosphorylation of key proteins in the insulin signaling [87–89]. By an oxidative inhibition of protein tyrosine phosphatase 1b, H_2O_2 may exert a pro-insulin or insulin-mimic action on phosphorylation of the β subunit of the insulin receptor in rat adipocytes [90]. In general, body insulin sensitivity is largely controlled by the balance between activities of protein kinases (phosphorylation) and protein phosphatases (de-phosphorylation). In the GPx1 overexpressing mice, insulin resistance was associated

with an attenuated phosphorylation of insulin receptor (β subunit) and AKT (Ser^{473} and Thr^{308}) after insulin stimulation in liver and muscle [40]. These decreased phosphorylations were presumably caused by the diminished intracellular ROS that lifted the oxidative inhibition of protein tyrosine phosphatases. In contrast, knockout of GPx1 resulted in enhanced phosphorylation of AKT in muscle [42]. Most interesting, knockout of GPx1 rendered mice resistant to a high-fat diet induced insulin resistance via an increased oxidation of the protein tyrosine phosphatase family member phosphatidylinositol 3-kinase phosphatase with tensin homology in muscle that terminates signals generated by phosphatidylinositol-3-kinase [91]. Reciprocally, the improvement was reversed by supplementing the antioxidant, *N*-acetylcysteine.

While importance of basal levels of ROS in insulin signaling is well illustrated in the above-discussed GPx1 overexpressing and knockout mice, outcomes may be totally different after prolonged exposure to high levels of ROS in diabetic subjects [92]. Another pathway that might also contribute to insulin resistance in the GPx1 overexpressing mice is their elevated body fat deposit. Limited experimental evidence has led to a postulation that high Se supply or high GPx1 activity may affect body lipogenesis via regulation of protein tyrosine phosphatase 1b [93].

20.4 GPx1 and Diabetic Complications

It is well accepted that oxidative stress is implicated in various diabetic complications: neuropathy [94–97], nephropathy [98–100], retinopathy [101], and vasculature and heart disease [102]. In screening 184 Japanese type 2 diabetic patients, variants in GPx1 gene Pro198Leu were found to be associated with increased intima-media thickness of carotid arteries and risk of cardiovascular and peripheral vascular diseases [15]. Supplemental antioxidants were beneficial to prevent or reverse diabetic complications [101]. A novel synthetic antioxidant with GPx-like activity reduced diabetes-associated-atherosclerosis in diabetic ApoE knockout mice [103].

20.5 Conclusions and Perspectives

Linking GPx1 overexpression to type 2 diabetes-like phenotypes reveals a novel role of GPx1 and creates a new field of Se biology, although the full role and the underlying mechanism are far from clear. With the “prevailing” perception of low antioxidant capacity in islet β cells and involvement of oxidative stress in pathogeneses of diabetes, both research and clinical scientists have unquestionably viewed upregulating islet or global antioxidant defense as an effective strategy to prevent and treat diabetes. In fact, many past studies have overly amplified transient benefits of antioxidant treatments against a bolus of ROS, but neglected long-term metabolic consequences of shifting cellular redox status. Demonstrating the type 2 diabetes-like phenotype in the GPx1 overexpressing mice provides a more realistic and

balanced concept of antioxidant enzymes in diabetes. Elucidating the effects of GPx1 overproduction on expression and(or) functions of PDX1, UCP2, p53 and protein tyrosine phosphatases unveils new in vivo regulation of pancreatic β cell mass and insulin physiology. These findings will help study etiology and potential risk associated with the pro-diabetic effects of Se supplements shown in recent human studies.

Overall, this chapter outlines the physiological importance and molecular mechanism for a dual role of the most abundant selenoprotein, GPx1, in diabetes. Clearly, maintaining the physiological level of ROS and a proper balance with GPx1 is essential to avoid dysregulation of islet integrity, insulin function, and glucose homeostasis. However, the desirable balance between ROS and antioxidant defense including GPx1 could differ greatly with diabetic status or at late stage of complications when target tissues or functions are exposed to high levels of ROS for an extended period.

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