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Tiam1/Vav2-Rac1 axis: A tug-of-war between islet function and dysfunction

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Abstract

Glucose-stimulated insulin secretion [GSIS] from the islet β -cell involves a well-orchestrated interplay between metabolic and cationic events. It is well established that intracellular generation of adenine and guanine nucleotide triphosphates [e.g., ATP and GTP] represents one of the requisite signaling steps in GSIS. The small molecular mass GTP-binding proteins [G-proteins; e.g., Rac1 and Cdc42] have been shown to regulate islet β -cell function including actin cytoskeletal remodeling and fusion of insulin granules with the plasma membrane for GSIS to occur. In this context, several regulatory factors for these G-proteins have been identified in the pancreatic β -cell; these include guanine nucleotide exchange factors [GEFs] and guanine nucleotide dissociation inhibitors [GDI]. Recent pharmacological and molecular biological evidence identified Tiam1 and Vav2 as GEFs for Rac1 in promoting physiological insulin secretion. Paradoxically, emerging evidence in multiple cell types, including the islet β -cell, suggests key roles for Rac1 in the onset of cellular dysfunction under conditions of metabolic stress and diabetes. Furthermore, functional inactivation of either Tiam1 or Vav2 appears to attenuate sustained activation of Rac1 and its downstream signaling events [activation of stress kinases] under conditions of metabolic stress. Together, these findings suggest both “friendly” and “non-friendly” roles for Tiam1/Vav2-Rac1 signaling axis in islet β -cell in health and diabetes. Our current understanding of the field and the knowledge gaps that exist in this area of islet biology are heightened herein. Furthermore, potential caveats in the specificity and selectivity of pharmacological inhibitors that are available currently are discussed in this *Commentary*.

Graphical Abstract

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Keywords

Islet β -cell; glucotoxicity; G proteins; Rac1; Tiam1; and Vav2

1. Glucose-stimulated insulin secretion [GSIS]: A simplified model

Insulin secretion from the pancreatic β -cell is regulated by the ambient concentrations of glucose in the interstitial fluid surrounding the islet cells. The stimulus-secretion coupling of glucose-stimulated insulin secretion [GSIS] is initiated by Glut-2-mediated entry of glucose into the β -cell followed by a series of metabolic and ionic events leading to an increase in the intracellular ATP/ADP ratio. This, in turn, results in the closure of ATP-sensitive potassium channels localized on the plasma membrane, resulting in membrane depolarization and the influx of extracellular calcium through membrane-associated voltage-gated calcium channels. A net increase in the influx of extracellular calcium into the cytosolic compartment, in addition to the mobilization of intracellular calcium from its storage pools [e.g., endoplasmic reticulum], are critical for the transport of insulin containing secretory granules to the plasma membrane for fusion and release of insulin into the circulation [1,2].

From a mechanistic standpoint, it is well established that GSIS is mediated largely *via* the generation of soluble second messengers, such as cyclic nucleotides, and hydrolytic products of phospholipases A₂, C, and D [1–4]. It is becoming increasingly clear that changes in intracellular calcium concentrations [as above] also regulate activities of various enzymes within the β -cell, including protein kinases, phosphodiesterases, adenylyl cyclases, and phospholipases leading to exocytotic secretion of insulin. In addition to regulation by adenine nucleotides [e.g., ATP], original investigations by Metz and associates have demonstrated contributory roles for guanine nucleotides [i.e., GTP] in GSIS. For example, using selective inhibitors of the GTP biosynthetic pathway [e.g., mycophenolic acid], they documented a permissive role for GTP in GSIS [5]. Although the precise mechanisms underlying the regulatory role[s] of GTP remain elusive, available evidence indicates that they might involve activation of one [or more] GTP-binding proteins [G proteins] endogenous to the islet β -cell [6–8].

2. G proteins in the pancreatic islet β -cell

At least three classes of G proteins have been described to date in β -cells. The first group is heterotrimeric in nature, and comprised of the α - [39–53 kDa], β - [~37 kDa], and γ - [7–10 kDa] subunits. Typically, trimeric G proteins are involved in coupling membrane-associated receptors [GPCRs] to their intracellular effectors [e.g., ion channels, phospholipases, adenylyl cyclases, and phosphodiesterases]. The second group is monomeric in nature [17–

30 kDa]; these G proteins regulate protein sorting and trafficking of secretory vesicles in many cell types. The third group of G proteins are the elongation factors and *Tau* proteins, which are involved in protein synthesis. Although these proteins are characterized extensively in other cell types, little is studied about the functional regulation of elongation factors in islet function, including physiological insulin secretion [6–8]. It is well established that G proteins cycle between their inactive [GDP-bound] and active [GTP-bound] conformations, which are tightly controlled by specific regulatory proteins/factors; this is often referred to as the GTP-hydrolytic cycle. Three major types of such regulatory proteins/factors have been described for small G proteins. The first group is comprised of guanine nucleotide exchange factors [GEFs], which promote the conversion of the GDP-bound [inactive] forms to their GTP-bound [active] forms. The second group is the GDP-dissociation inhibitors [GDIs], which prevent the dissociation of GDP from G proteins, and hence are considered negative modulators of the G protein activation cascade. The third group represents the GTPase-activating proteins [GAPs]; these proteins promote the conversion of the GTP-bound G proteins to their GDP-bound [inactive conformation] to complete the GTP hydrolytic cycle [7,8]. For the sake of brevity, this article is focused primarily on the currently available evidence on the identity, subcellular distribution, functional activation, and potential roles of Rac1 and its regulatory proteins/factors in the function of the pancreatic β -cell in health and diabetes. As depicted in Figure 1, Rac1 undergoes activation-deactivation [GTP-hydrolytic] cycle. The GDP-bound inactive Rac1 is converted to its active, GTP-bound conformation by GEFs, including T-lymphocyte invasive and metastasis protein 1 [Tiam1] and the guanine nucleotide exchange factor Vav2 [Vav2]. Published evidence suggests that both Tiam1 and Vav2 are expressed in a variety of insulin-secreting cells including clonal INS-1 832/13 cells, rodent islets and human islets [9,10]. Using small molecule inhibitors for Tiam1 [e.g., NSC23766] and Vav2 [e.g., Ehop-016] recent studies have demonstrated critical regulatory roles for these GEFs in islet function in health and metabolic stress [see below]. This article thus represents an update of our current understanding of contributory roles of Tiam1 and Vav2 in mediating the functional activation of Rac1 and the phagocyte-like NADPH oxidase [Nox2] in the islet β -cell in health and diabetes.

3. Phagocyte-like NADPH oxidase [Nox2] in the pancreatic islet β -cell

NADPH oxidases represent the principal source of cellular reactive oxygen species [ROS]. In this category of enzymes, the Nox2 holoenzyme [also referred to as the respiratory burst] has been implicated in phagocytosis by professional phagocytic cells including neutrophils, eosinophils, monocytes and macrophages. The Nox2 holoenzyme is a highly regulated membrane-associated protein subunit complex that mediates the catalysis of one-electron reduction of oxygen to superoxide anion involving oxidation of cytosolic NADPH, thereby generating excessive amounts of intracellular ROS, which, in turn, promote killing of phagocytized microorganisms, including fungi and a variety of bacteria. It has been demonstrated that defective activation of Nox2 leads to the pathology of disease states such as the chronic granulomatous disease in humans [11,12]. Several plausible mechanisms have been put-forth for the generation and involvement of ROS in a variety of non-phagocytic cell types, including the islet β -cell [11,12]. For example, emerging evidence from *in vitro* and *in*

in vivo studies provides strong support to the hypothesis that a tonic increase in ROS is necessary for cytoskeletal remodeling [13] and secretion of insulin under physiological conditions [7,14–16]. Evidence is also accumulating to suggest that chronic exposure of β -cells to elevated glucose [glucotoxicity], saturated fatty acids [lipotoxicity] or glucose plus lipids [glucolipotoxicity] results in sustained increase in ROS generation [oxidative stress] culminating in metabolic dysregulation eventually leading to cell demise [7,11,12,17,18]. Multiple mechanisms have been proposed in this context, including depletion of intracellular redox equivalents *via* the oxidation of reducing equivalents [e.g., reduced glutathione] and activation of superoxide-generating enzymatic machinery. One of the enzymatic steps involved in the increased generation of ROS and associated induction of intracellular oxidative stress in the pancreatic β -cell includes activation of Nox2 system [11,12]. Briefly, Nox2 is a highly regulated membrane-associated protein complex that catalyzes the one electron reduction of oxygen to superoxide anion involving oxidation of cytosolic NADPH [Figure 2]. Nox2 is a multicomponent system comprised of membrane as well as cytosolic cores of proteins. The membrane-associated catalytic core is comprised of gp91^{phox}, p22^{phox} and the small G protein Rap1. The cytosolic regulatory components include p47^{phox}, p67^{phox} and the small G protein Rac1. Following stimulation, the cytosolic components of Nox2 translocate to the membrane fraction for association with the catalytic core for holoenzyme assembly. For example, phosphorylation of p40^{phox}, p47^{phox} and p67^{phox} is considered to be an important signaling step for translocation of the cytosolic core. Available evidence suggests that a protein kinase C-sensitive phosphorylation of p47^{phox} leads to its translocation to the membrane fraction [20]. It is noteworthy that recent site-directed mutagenesis studies by Teng et al [21] have demonstrated differential regulatory roles of p47^{phox} on TNF α signaling in endothelial cells. Their findings implicated double serine [Serine 303–4] phosphorylation is requisite for TNF α -induced [acute; 30 min] O(2)(-) production. Interestingly, phosphorylation at these residues is not essential for TNF α signaling cascade through TNF receptor-associated factor 4 and ERK1/2 phosphorylation. Based on these findings, these investigators concluded that TNF α signaling module requires phosphorylation of p47^{phox} at distinct serine residues [21]. Along these lines, Mohammed and coworkers [22] have reported significant upregulation of Nox2 activity by proinflammatory cytokines [a mixture of IL-1 β , TNF α and IFN γ ; cytomix] in insulin-secreting β -cells. These studies demonstrated time-dependent [0–60 min] induction of p47^{phox} phosphorylation by the cytomix. Based on these findings they concluded that Nox2-mediated generation of intracellular oxidative stress plays key regulatory roles in the metabolic dysfunction of the islet β -cell [22; see *section 7.3* below for additional discussion]. In addition to phosphorylation of the p22^{phox}, p47^{phox} and p67^{phox} subunits as a trigger for translocation of the cytosolic core, it has also been shown that functional activation of Rac1 [Rac-GTP], mediated by GEFs [Tiam1 and Vav2] is vital for the holoenzyme assembly and activation of Nox2. Based on evidence from a large number of recent studies, it has been suggested that Nox2 plays critical regulatory roles in islet β -cell function in health and diabetes [7,11,12,14–18]. Furthermore, data accrued in studies involving islets from animal models of type 1 and type 2 diabetes have identified Nox2 as one of the potential drug targets for preventing oxidative damage to the islet [see below].

4. Regulatory roles of Rac1 in islet function including physiological insulin secretion

Original investigations involving bacterial toxins, which irreversibly monoglucosylate and inactivate specific small G proteins, suggested requisite roles for Rac1 in physiological insulin secretion [7,8,23]. Li and associates [24] further demonstrated essential roles for Rac1 in GSIS. For example, they reported that exposure of insulin-secreting INS-1 832/13 cells to stimulatory glucose concentrations resulted in *transient* activation [~15 min] followed by translocation of cytosolic Rac1 to the plasma membrane. Further evidence for a key regulatory role for Rac1 in GSIS was obtained using a dominant-negative mutant of Rac1 [N17Rac1]. Expression of N17Rac1 in INS-1 832/13 cells resulted in significant morphological changes and disappearance of F-actin structures leading to vastly compromised GSIS. Subsequent studies using specific small molecule inhibitors of post-translational geranylgeranylation of Rac1 [e.g., GGTI-2147] or overexpression of an inactive mutant of the regulatory α -subunit of prenyltransferase, which catalyzes the geranylgeranylation of Rac1, demonstrated selective accumulation of unprenylated in the cytosolic fraction culminating in reduced GSIS in pancreatic β -cells [25]. It is noteworthy that siRNA-Rac1 markedly suppressed GSIS in INS-1 832/13 cells [18]. In further support of these *in vitro* observations Asahara *et al.* reported impaired glucose tolerance and hypoinsulinemia in Rac1-null [β Rac1^{-/-}] mice. Furthermore, GSIS was also significantly decreased in islets from β Rac1^{-/-} mice [26]. In summary, data accrued from studies involving pharmacological and molecular biological tools as well as knockout animal models provide compelling evidence for regulatory role for Rac1 in islet function, including GSIS. For additional information on this topic, the reader is referred to a recent *Commentary* highlighting the friendly and non-friendly roles of Rac1 in islet function in health and diabetes [18]. Existing evidence on the identification and characterization of two GEFs, namely Tiam1 and Vav2, in the functional regulation of Rac1 in pancreatic β -cells is highlighted in the following section.

5. Regulatory roles of Tiam1-Rac1 axis in physiological insulin secretion

It is becoming increasingly clear that activation of Rac1 is mediated by a variety of GEFs including Tiam1, Vav2 and Trio [9,10,27]. Gao and associates discovered a small molecule compound, NSC23766, that specifically inhibited Tiam1 or Trio-induced activation of Rac1 [27]. Specificity studies indicated that activation of other small G-proteins [Cdc42 and Rho] was resistant to NSC23766. This inhibitor also prevented Rac1-induced proliferation, anchorage-independent growth and invasion phenotypes in human prostate cancer PC-3 cells [27]. Since its discovery in 2004, nearly 300 studies utilized NSC23766 as an inhibitor for Rac1 activation. Using NSC23766 we reported [9] a significant inhibition of glucose-induced activation of Rac1, but not Cdc42 or Rho in pancreatic β -cells further confirming its specificity to inhibit Tiam1-Rac1 signaling axis. It also inhibited glucose-induced membrane association of Rac1 and GSIS in a variety of insulin-secreting cells. In further support of pharmacological data, siRNA-Tiam1 markedly inhibited glucose-induced membrane trafficking and activation of Rac1 in INS-1 832/13 cells. Based on the immunological, pharmacological and molecular biological data, we surmised that Tiam1 represents one of

the GEFs for Rac1 in the pancreatic β -cell, and that Tiam1-Rac1 signaling pathway is involved in physiological insulin secretion [9].

6. Regulatory roles of Vav2-Rac1 axis in physiological insulin secretion

Using immunological, molecular biological, pharmacological and microscopic approaches recent studies have investigated regulatory roles of Vav2 in GSIS from islet β -cells [10]. Immunological evidence indicated that Vav2 is expressed in INS-1 832/13 β -cells, normal rat islets and human islets. Vav2-siRNA markedly reduced glucose-induced Rac1 activation and GSIS in INS-1 832/13 cells. Furthermore, Ehop-016, a small molecule inhibitor of Vav2-mediated activation of Rac1, significantly attenuated GSIS in INS-1 832/13 cells and primary rat islets [10]. It is noteworthy that stimulatory glucose concentrations promoted association [co-localization] of Rac1 and Vav2 further supporting the pharmacological and molecular biological evidence to suggest significant cross-talk between Vav2-Rac1 under conditions of physiological insulin secretion. Lastly, real-time imaging in live β -cells indicated a marked reduction in glucose-induced cortical actin remodeling following inhibition of Vav2-mediated activation of Rac1 by Ehop-016. Based on these findings, the authors proposed novel roles for Vav2 in glucose-induced Rac1 activation, actin remodeling and GSIS in pancreatic β -cells [10].

Taken together, it is evident that GSIS involves *transient* activation of Rac1, which is mediated by Tiam1 and Vav2. Such conclusions were drawn by pharmacological, molecular biological, immunological and microscopic evidence in a variety of insulin-secreting cells including INS-1 832/13 cells, primary rat islets and human islets. Interestingly, exposure of isolated β -cells to a variety of pathological conditions *in vitro* [e.g., high glucose, high palmitate, proinflammatory cytokines or biologically-active sphingolipids, such as ceramide] has been shown to promote sustained activation of Rac1 resulting in metabolic dysregulation of the effete β -cell culminating in loss of GSIS, mitochondrial and nuclear abnormalities and apoptotic demise [7,17,28]. These *in vitro* observations were replicated in animal models of type 2 diabetes [ZDF rat] and type 1 diabetes [NOD mouse] and in islets derived from human donors with type 2 diabetes [see below]. It is noteworthy that both Tiam1 and Vav2 appear to contribute to sustained activation of Rac1 and damage to the β -cell under above conditions. The following section provides an update on our current understanding of potential roles of Tiam1/Vav2-Rac1 signaling axes on islet dysfunction induced under the duress of a variety of experimental conditions including glucotoxicity, lipotoxicity and exposure of proinflammatory cytokines.

7. Regulatory roles of Tiam1-Rac1 axis in the induction of islet dysfunction

7.1. Islet dysfunction induced by glucotoxic conditions

It is well established that glucotoxicity underlies pathogenesis of islet dysfunction in diabetes. As described above, Nox2 appears to play a significant regulatory role in the generation of reactive oxygen species [ROS] under high glucose [HG; glucotoxicity] conditions, resulting in mitochondrial dysregulation and loss of islet β -cell function. Recent studies examined roles of Nox2 as a regulator of downstream stress kinase [p38MAPK] activation under glucotoxic conditions in clonal INS-1 832/13 cells and primary rat islets.

Data from these studies revealed that *gp91-ds-tat*, a specific inhibitor of Nox2, but not its inactive analog, markedly reduced HG-induced Nox2 activation, ROS generation and p38MAPK activation, thus suggesting that Nox2 activation couples with p38MAPK activation [29]. Germane to this article are observations indicating that inactivation of Tiam1-Rac1 axis [NSC23766] significantly reduced HG-induced p38MAPK activation in isolated β -cells; these data implicated novel regulatory roles for Tiam1-Rac1 module in the activation of specific stress kinase [p38MAPK] in HG-mediated Nox2-dependent β -cell dysfunction [29]. It should be noted that Tiam1-Rac1 signaling also contributes to islet dysregulation induced by lipotoxic conditions as highlighted in the following section.

7.2. Islet dysfunction induced by lipotoxic conditions

Using normal rat islets and clonal INS-1 832/13 cells, Syed and coworkers tested the hypothesis that Tiam1-Rac1 pathway contributes to Nox2-mediated dysfunction of the pancreatic beta-cell under the duress of lipotoxic conditions [30]. They demonstrated that incubation of isolated β -cells with palmitic acid, a saturated fatty acid, known to induce cell dysfunction [lipotoxicity] increased the Nox2 activity leading to an increase in the generation of superoxides and lipid peroxides in these cells; such effects of palmitate were attenuated by diphenyleneiodonium, a known inhibitor of Nox2, thus implicating Nox2 in cellular dysregulation induced by palmitate. Under these conditions, palmitate-induced Rac1 activation, superoxide and lipid peroxide generation were inhibited by NSC23766; these data implicate Tiam1-Rac1 in palmitate-induced effects on isolated β -cells. Additional studies suggested that palmitate-induced generation of superoxides and lipid peroxides was reduced by fumonisins B1, a known inhibitor of ceramide biosynthesis from palmitate suggesting that intracellular generation of ceramide could contribute to palmitate-induced metabolic dysregulation of the beta-cell. In further support of this formulation, NSC23766 prevented C2-ceramide [a cell permeable analog of ceramide]-induced Rac1 activation and production of superoxides and lipid peroxides. Lastly, cell-permeable C2-ceramide, but not its inactive analogue, significantly reduced the mitochondrial membrane potential, which was prevented to a large degree by NSC23766. These observations led to the proposal that Tiam1-Rac1 signaling pathway mediates palmitate-induced, ceramide-dependent superoxide generation and mitochondrial dysfunction in pancreatic β -cells [30].

7.3. Islet dysfunction induced by proinflammatory cytokines

In addition contributory roles of Tiam1-Rac1 in β -cell dysfunction induced under the duress of glucotoxic and lipotoxic conditions, experimental evidence also implicates key regulatory roles for this signaling module in the metabolic abnormalities induced by proinflammatory cytokines. Observations from these investigations are highlighted below.

Original studies by Subasinghe and coworkers [31] have demonstrated that exposure of insulin-secreting INS-1 832/13 cells to a mixture of proinflammatory cytokines [IL-1 β , IFN- γ , and TNF- α ; for 24 hrs.] resulted in a significant, time-dependent increase in Nox2 activity and associated intracellular ROS production. An increase in the expression of p47^{phox} subunit, but not p67^{phox} subunit, was also seen under these conditions. Transfection of INS-1 832/13 cells with siRNA-p47^{phox} or exposure to apocynin, a known inhibitor of Nox2, markedly suppressed cytokine-induced ROS generation in these cells. Inhibition of

Tiam1-Rac1 signaling pathway [NSC23766] not only attenuated the cytomix-induced Rac1 activation but also prevented loss of mitochondrial membrane potential induced by commix. Based on these observations, Subasinghe and associates proposed that Tiam1-Rac1-mediated regulation of Nox2 contributes to cytokine-mediated mitochondrial dysfunction in the β -cell [31].

As a logical extension to the aforesaid investigations *in vitro*, a recent study assessed the contributory roles for Tiam1-Rac1 signaling pathway in the onset of spontaneous diabetes in the nonobese diabetic [NOD] mouse, an accepted model for type-1 diabetes [32]. To assess this Balb/c mice [control animals] or NOD mice were treated with either saline or NSC23766. Body weights and blood glucose were measured in these animals every week for 34 weeks. Findings from these studies indicated that administration of NSC23766 significantly prevented the development of spontaneous diabetes in the NOD mice. Furthermore, NSC23766 markedly suppressed Rac1 expression and activity and the endoplasmic reticulum stress in NOD mouse islets. Together, based on data accrued in *in vitro* and *in vivo* experiments, the authors proposed a key regulatory roles for Tiam1-Rac1-Nox2 signaling pathway in the islet dysfunction and demise induced by proinflammatory cytokines.

8. Regulatory roles for Vav2-Rac1 signaling pathway in islet dysfunction

A recent study assessed whether the Vav2-Rac1 signaling module mediates HG-induced stress kinases [p38MAPK] activation in pancreatic β -cells. To accomplish this, the investigators utilized Ehop-016, a specific inhibitor of Vav2-mediated activation of Rac1. Data from these investigations in INS-1 832/13 cells and primary rat islets indicated a significant inhibition of p38MAPK activation by Ehop-016 under glucotoxic conditions. It is noteworthy that Ehop-016 exerted stimulatory effects on p38MAPK in normal rat islets and INS-1 832/13 cells under basal glucose exposure conditions while inhibiting HG-induced p38MAPK [29]. The reasons for such effects of this Rac1 inhibitor on basal p38MAPK appear to be unknown at this time. It was speculated that such effects might include activation of other Rho subfamily of G-proteins (*e.g.*, Rho) as demonstrated by Montalvo-Ortiz and associates recently [33]. However, additional studies are needed to further address these findings.

Data from the above studies collectively suggest novel regulatory roles of Tiam1 and Vav2 in Rac1-mediated activation of p38MAPK in pancreatic β -cells exposed to glucotoxic conditions. This represents the first evidence in support of involvement of more than one GEF in modulation of G-protein [Rac1]-mediated metabolic dysfunction of the islet β -cell. It is speculated that these two GEFs might mediate HG-induced activation of Rac1-sensitive Nox2 [see Working Model; Figure 3 below].

9. Working model

Based on the above discussion, a working model [Figure 3] is proposed, which states that under physiological conditions, glucose promotes activation of Rac1 [$\sim$ 15 minutes], which is mediated by Tiam1 and/or Vav2 as evidenced by inhibition of GSIS by specific inhibitors of

Tiam1-Rac1 [NSC23766] and Vav2-Rac1 [Ehop-016], respectively. As discussed above, data from multiple laboratories have demonstrated activation of Nox2 under these conditions resulting in *transient* increase in intracellular ROS generation and associated increase in ROS generation. It has also been suggested that tonic increase in ROS may promote cytoskeletal remodeling, which is requisite for translocation of insulin-laden secretory granules to the plasma membrane for fusion with plasma membrane for exocytotic secretion of insulin. On the other hand, chronic exposure of pancreatic β -cells to glucotoxic conditions results in sustained activation of Rac1, also mediated by Tiam1 and/or Vav2 leading to increased oxidative stress due to sustained activation of Nox2. Recent pharmacological evidence also demonstrated activation of stress kinases [p38 MAPK, p53 and JNK1/2] under these conditions leading to mitochondrial dysfunction, caspase activation and cell demise [17,29]. Furthermore, recent reports have demonstrated significant alterations in the nuclear compartment [increased caspase-mediated degradation of lamins] under these conditions leading to cell demise [28,34].

10. Do Tiam1 and Vav2 work together to facilitate Rac1-mediated cell function?

The above model [Figure 3] suggests that both Tiam1 and Vav2 are necessary for physiological insulin secretion as well as in the induction of metabolic dysfunction induced by glucotoxic, lipotoxic and diabetic conditions. These findings suggest a model that both Vav2 and Tiam1 could contribute to Rac1 activation and cellular [dys]function at multiple levels, including regulation in specific subcellular compartments [e.g., cytosol and plasma membrane]. In this context, published evidence in multiple cell types including the islet β -cell provides further support for such a possibility [summarized in Table I]. For example, Price and associates in COS^{phox} cells demonstrated significant translocation of cytosolic components of Nox2 holoenzyme, namely p47^{phox} and p67^{phox} to the membrane fraction following expression of constitutively active Rac1 [35]. Furthermore, activation of endogenous Rac1 by overexpression of constitutively-active Tiam1 or Vav1 resulted significant activation of Nox2-mediated superoxide generation. It is important to note that overexpression of Vav1 exerted more pronounced effects on superoxide generation compared to cells overexpressing constitutively active Tiam1. Based on these observations, the authors proposed dual regulatory roles of Rac1 in the activation of Nox2; first by directly participating in the Nox2 complex as a member of the holoenzyme and second by promoting the activation of signaling events leading to oxidase assembly. Lastly, they also highlighted key roles for Vav1 as the physiological relevant GEF responsible for the activation of Rac1 regulated Nox2 complex [35].

Recent findings in the pancreatic islet gain further support from recent reports of Liu et al [36] suggesting dual regulation of Rac1 by Tiam1 and Vav2 in eliciting activation of Nox2. These investigators discovered a novel polarity complex that directs localized Rac1 activation required for downstream ROS production. They also demonstrated that Vav2 is essential for the attainment of the active conformation of Rac1 [Rac1-GTP], whereas, Tiam1 functions as an adaptor in a VE-cadherin-p67^{phox}-Par3 polarity complex that directs localized activation of Rac1. They also demonstrated that compromised Tiam1 function

culminates in the disruption of redox signaling both *in vitro* and *in vivo* [36]. Based on these findings, these authors concluded that both Vav2 and Tiam1 mediate regulation of Rac1 by linking components of the polarity complex to NADPH oxidase.

Peotter and associates [37] have recently assessed potential roles of Tiam1 and Vav2 in the regulation of Rac1-mediated phagocytosis in human trabecular meshwork [TM] cells. Using pharmacological and molecular biological approaches they demonstrated that $\alpha_v\beta_5$ integrin/FAK-mediated phagocytosis in these cells is mediated by Rac1. Interestingly, however, while Ehop-016, a known inhibitor of Vav2, inhibited phagocytosis, siRNA-mediated depletion of Vav2 failed to inhibit phagocytosis. Moreover, siRNA-mediated knockdown of Tiam1, but not pharmacological inhibition of Tiam1-Rac1 signaling pathway [NSC23766] markedly suppressed phagocytosis. These studies have also identified ELMO2, RhoG and ILK as upstream regulators of Rac1 activation. Based on these observations, the authors concluded that Tiam1, but not Vav2, plays key regulatory roles in promoting Rac1-mediated phagocytosis in TM cells [37].

There may be additional regulatory mechanisms that underlie the dual regulation of Rac1 by Tiam1 and Vav2. For example, Arthur and coworkers demonstrated critical roles for Rap1 in integrin-mediated adhesion and spreading in various mammalian cell types. These researchers demonstrated that Rap1 regulates cell spreading by precisely modulating the subcellular localization of GEFs for Rac1 [38]. They suggested requisite roles for Rac1 in Rap1-mediated effects including enhanced cell spreading. Interestingly, however, Rac1 induced cell spreading independent of Rap1. Active Rap1a binds to a subset of Rac GEFs, including Vav2 and Tiam1 but not others such as SWAP-70 or COOL-1. Overexpressed Vav2 and Tiam1 specifically require Rap1 to promote spreading, even though Rac1 is activated independently of Rap1. Based on these and other data accrued in these studies, the authors proposed that Rap1 promotes cell spreading by localizing a subset of Rac GEFs to sites of active lamellipodia extension.

It may be germane to point out in this context that while Rac1 has been shown to be integral to Nox2 holoenzyme assembly and activation [above], very little is known on the contributory roles of Rap1 in the regulation of Nox2 activity and the onset of oxidative stress in the pancreatic β -cell. Original contributions from the author's laboratory have provided evidence that Rap1 activation is necessary for GSIS to occur [23,39]. Earlier studies by Leiser and associates [40] have demonstrated that Rap1 undergoes post-translation methylation at its carboxylterminal cysteine, and established direct correlation between the carboxylmethylation of Rap1 and insulin secretion in β -TC3 cells. Kelly et al [41] have further demonstrated novel regulatory roles for Rap1 in GSIS, islet cell hypertrophy and islet cell proliferation. Together, findings from these studies suggest that Rap1 is an important regulator of islet β -cell function. It is noteworthy that exposure of pancreatic β -cells to glucotoxic conditions resulted in selective accumulation of unprenylated Rap1 suggesting significant abnormalities in Rap1 function under these conditions [42]. However, potential significance of accumulation of unprenylated Rap1 to the onset of metabolic dysfunction of the islet, including loss in GSIS and sustained activation of Nox2, remains to be verified further.

11. Potential caveats that we need to consider in further validating our model

Published evidence in multiple cell types indicates pleotropic effects of NSC23766. For example, Suzuki et al [43] have demonstrated that both apocynin [a known inhibitor of Nox2] and NSC23766 inhibited cell proliferation and phosphorylation of Rac1, NF- κ B and cyclin D1 in prostate cancer cells. These findings suggest potential off-target effects of NSC23766 on cell function since effects of NSC23766 are known to be mediated primarily through inactivation of Tiam1-mediated activation of Rac1. However, the inhibitory effects of NSC23766 on phosphorylation of Rac1 were studied using relatively high concentration of the inhibitor [200 μ M], which is at least 10-fold higher than what is required for inhibition of Tiam1-Rac1 signaling steps [$\sim$ 20 μ M]. Published evidence also suggests that NSC23766 elicits competitive antagonistic effects on muscarinic acetylcholine receptors. For example, Levay et al. recently reported inhibition of M2 muscarinic acetylcholine-induced Rac1 activation by NSC23766 in neonatal rat cardiac myocytes [44]. Interestingly, NSC23766 [50 μ M] also inhibited carbachol-induced RhoA activation and muscarinic acetylcholine-induced inotropic response in isolated neonatal rats requiring the activation of Rho-dependent kinases. More recent studies by Dutting and associates [45] have reported critical off-target effects of NSC23766 and EHT1864 [a known inhibitor of Rac1; ref. 46] in mouse platelets. They demonstrated distinct Rac1-independent effects of these inhibitors on platelet spreading. More importantly, these inhibitors markedly altered platelet function in Rac1-null platelets. These findings raise important concerns about the specificity of these inhibitors. However, as described above, such off-target effects of these inhibitors were noted at relatively high concentrations [50–100 μ M] of these inhibitors than those typically required for inhibition of Rac1 functions [see above]. Together, these data suggest potential non-specific, Rac1-independent effects of NSC23766, specifically when used in higher concentrations.

It is noteworthy that while there is a general agreement with regard to potential regulatory roles of Nox2 in islet function in health and diabetes, recent studies by de Souza and coworkers have provided additional insights into potential regulatory roles of Nox2 in islet dysfunction [47]. Using islets derived from Nox2 global knockout mice and the wild-type C57BL/6 mice, these authors demonstrated that Nox2 is a negative modulator of GSIS, and that such an effect is independent from alterations in cytosolic redox state [thiol and glutathione status]. Furthermore, they also proposed that Nox2 activation is not involved in β -cell dysfunction and demise under the duress of long-term exposure to hyperglycemic conditions. While there is a general agreement that alterations in intracellular redox status and generation of oxidative stress are causal for islet β -cell dysregulation under conditions of glucotoxicity and lipotoxicity [above], they rule out the contributory roles for Nox2 in these signaling events culminating in cellular apoptosis. As appropriately pointed out by the authors as a note of caution in their publication, additional studies are needed to further validate such a formulation including quantification of intracellular redox state induced by glucotoxic conditions in islets derived from animal models in which components of holoenzyme of Nox2 are conditionally deleted in the islet β -cell [47].

Lastly, it should be kept in mind that functional activation and subcellular targeting of Rac1 has been shown to be under the control of post-translational modifications including geranylgeranylation, palmitoylation, phosphorylation, ubiquitination and SUMoylation [48,49]. It has also been reported recently that inhibition of geranylgeranylation results in constitutive or sustained activation of Rac1; such a signaling step has been implicated in the sustained activation of Rac1 in the pancreatic β -cell exposed to metabolic stress conditions leading to increased oxidative stress due to chronic activation of Nox2-ROS signaling pathway [42]. Lastly, published evidence suggests expression and regulation of a variety of GEFs [nearly 20–30; refs. 49,50] that can mediate functional activation of Rac1. Expression of these GEFs in the islet β -cell, and their potential regulation of Rac1 and associated islet function remain to be investigated.

12. Conclusions and future directions

In conclusion, the small G protein Rac1 plays a regulatory role in GSIS in the pancreatic β -cell. Published evidence also supports involvement of Tiam1 and Vav2 as mediators of Rac1. It is proposed that *transient* activation of Rac1 by physiological glucose concentrations leads to [among other metabolic events] activation of Nox2 and associated generation of ROS, which, in turn, promote cytoskeletal remodeling, insulin granule transport and vesicle fusion and insulin secretion. However, long term exposure of the β -cell to pathological conditions such as glucotoxicity results in sustained activation of Tiam1/Vav2-Rac1 signaling module leading to constitutive activation of Nox2 and generation of intracellular oxidative stress, stress kinase activation, mitochondrial and nuclear alterations leading to apoptotic demise of the islet β -cell. Further, accumulating evidence implicates other physiological insults including lipotoxicity [ceramide-mediated?] or chronic inflammation [proinflammatory cytokines] could mediate their cytotoxic effects via activation of Tiam1/Vav2-Rac1-Nox2 pathway. Together, these observations identify Tiam1-Rac1-Nox2 as one of the therapeutic targets for prevention or halting metabolic defects induced by various pathological conditions. It should be noted however that while there is substantial evidence to support this viewpoint, some aspects remain speculative and needed to be confirmed experimentally. For example, considerable work is still needed to explain potential cross-talk and/or interdependence, if any, between Tiam1 and Vav2 in mediating their effects on Rac1 activation. In addition, potential regulatory mechanisms upstream to activation of these two GEFs need to be determined. It is also necessary to precisely determine the subcellular compartmentalization of these two GEFs under acute stimulatory conditions leading to GSIS and chronic exposure conditions culminating in cellular dysfunction and demise. As discussed above, it must be kept in mind that Rap1, which represents one of the components of the membranous core of Nox2 [Figure 2], could contribute significantly in the subcellular-localization [e.g., plasma membrane] of these GEFs in the steps leading to activation of Rac1 and optimal assembly of the Nox2 complex. Finally, evidence from the *in vitro* experiments appear to be in agreement with those accrued in islets derived from the diabetic animal models as evidenced by sustained activation of Rac1 in animal models of type 1 and type 2 diabetes. Functional relevance of GEF [Tiam1/Vav2] induced Rac1 activation in the generation of oxidative stress, stress kinase activation and mitochondrial

dysfunction need to be validated further in islets from animal models and human donors with diabetes.

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None declared

Abbreviations used

HG	High glucose
GEFs	Guanine nucleotide exchange factors
GSIS	Glucose-stimulated insulin secretion
NOD mice	Nonobese diabetic mice
Nox2	Phagocyte-like NADPH oxidase
Rac1	Ras-related C3 botulinum toxin substrate 1
ROS	Reactive oxygen species
Tiam1	T-lymphocyte invasive and metastasis protein 1
and Vav2	Vav guanine nucleotide exchange factor 2

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Figure 1. Schematic representation of activation-deactivation cycle of Rac1: Roles of Tiam1 and Vav2 as GEFs for Rac1 activation

It is well established that G proteins undergo activation [GTP-bound conformation] and deactivation [GDP-bound conformation]. Shown here is the activation-deactivation [GTP hydrolytic] cycle of Rac1. GDP-bound Rac1 [inactive] is converted to its active [GTP-bound] form by at least two GEFs, namely Tiam1 and Vav2. Published evidence implicates that both Tiam1 and Vav2 are expressed in a variety of insulin-secreting cells including clonal INS-1 832/13 cells, rodent islets and human islets. NSC23766 and Ehop-016 inhibit Tiam1 and Vav2, respectively [see text for additional details]. GTP-bound Rac1, in turn, regulates functions for multiple effector proteins, including Nox2. Following transmission of requisite signals, the active form of Rac1 is converted back to its inactive conformation mediated by the GTPase activity that is intrinsic to the G protein. It has been shown that additional regulatory proteins/factors, namely the GTPase-activating protein [GAP], further facilitate the conversion of the active G protein to its inactive form. The identity of GAP for Rac1 is an understudied area in the islet β -cell.

Figure 2. Schematic representation of assembly and activation of NoX2

Nox2 holoenzyme consists of cytosolic and membrane-associated components. The cytosolic core includes p67^{phox}, p47^{phox}, p40^{phox} and the small G-protein, Rac1. The membrane core is comprised of gp91^{phox}, p22^{phox}, and the small G protein, Rap1. Upon activation, Rac1.GDP [inactive] is converted to Rac1.GTP [active], which binds to p67^{phox}, and the complex translocates to the membrane. Existing evidence in other cell types suggests that phosphorylation of p47^{phox} also triggers its translocation to the membrane to form the Nox2 holoenzyme complex that culminates in the enzyme activation and associated increase in ROS. As depicted, Rap1, another small G protein is also associated with the membrane fraction. However, its regulatory role in Nox2 activation still remains an open area of investigation. It is likely that Rap1 plays key roles in the localization of Tiam1 and Vav2 in appropriate locations within the membrane for optimal activation of Rac1-mediated regulation of Nox2 assembly and activation [see text for additional discussion].

Figure 3. Our proposed model for dual regulatory roles for Tiam1/Vav2-Rac1 signaling axis in islet function and dysfunction

We propose that under physiological conditions, glucose promotes activation of Rac1 [~15 minutes], which is mediated by Tiam1 and/or Vav2 as evidenced by inhibition of GSIS by specific inhibitors of Tiam1-Rac1 [NSC23766] and Vav2-Rac1 [Ehop-016]. Data from multiple laboratories have demonstrated activation of Nox2 under these conditions resulting in transient increase in intracellular ROS generation and associated increase in ROS generation under these conditions. It has also been suggested that tonic increase in ROS may promote cytoskeletal remodeling, which is requisite for translocation of insulin-laden secretory granules to the plasma membrane for fusion and exocytotic secretion of insulin. On the other hand, chronic exposure of pancreatic β -cells to glucotoxic conditions results in sustained activation of Rac1, also mediated by Tiam1 and/or Vav2 leading to increased oxidative stress due to sustained activation of Nox2. Evidence is also emerging to support activation of stress kinases [p38 MAPK, p53 and JNK1/2] under these conditions leading to mitochondrial dysfunction and caspase activation. Furthermore, recent studies have reported significant alterations in the nuclear compartment [increased caspase-mediated degradation of lamins] under these conditions leading to cell demise [see text for additional details]. Please note that while the description provided in this figure highlights critical regulatory roles of Tiam1/Vav2-Rac1 in islet β -cell dysfunction under the duress of glucotoxicity, it is also evident that such a pathway is relevant for models of lipotoxicity and inflammation [e.g., exposure to proinflammatory cytokines].

Table I

Summary of studies depicting regulatory roles for Tiam1/Vav2-Rac1 in cell function

Findings [references]
NSC23766, a known inhibitor of Rac1 function inhibits glucose-induced Rac1 activation, membrane association and insulin secretion in clonal INS-1 832/13 cells and rodent islets. NSC23766 failed to inhibit KCl-induced insulin secretion. siRNA-mediated depletion of Tiam1 inhibited glucose-induced membrane trafficking and activation of Rac1 in INS-1 832/13 cells. These studies provided the first evidence to implicate Tiam1-Rac1 signaling in physiological insulin secretion [9].
Ehop-016, a known inhibitor of Vav2, suppresses glucose-induced Rac1 activation, cytoskeletal remodeling and GSIS in clonal INS-1 832/13 cells and rodent islets. These studies provided the first evidence to implicate Vav2 in physiological insulin secretion [10].
NSC23766 and Ehop-016 inhibit activation of stress kinases [p38MAPK] in INS-1 832/13 cells. These studies concluded that Rac1-Nox2 signaling module plays novel regulatory roles in HG-induced p38MAPK activation and loss in GSIS culminating in the metabolic dysfunction of the islet β -cell and onset of diabetes [29].
NSC23766 prevents Rac1 activation and cell dysfunction in pancreatic β -cells exposed to chronic gluco-, or lipotoxic conditions or exposure to ceramide and proinflammatory cytokines [18,30,31].
Administration of NSC23766 significantly prevents the onset of spontaneous hyperglycemia in the NOD mouse. NSC23766 markedly suppressed Rac1 expression and activation as well as endoplasmic reticulum stress in NOD islets [32].
Expression of constitutively active Rac1 promotes translocation of p47 ^{phox} and p67 ^{phox} to the membrane fraction in COS ^{phox} cells. Furthermore, overexpression of constitutively-active Tiam1 or Vav1 results in Rac1-mediated Nox2 activation and superoxide generation. Rac1 plays a dual role in Nox2 activation both by functioning as a member of the Nox2 holoenzyme as well as regulating the signaling steps requisite for Nox2 assembly [35].
Tiam1 and Vav2 mediate spatiotemporal activation of Rac1 and Nox2 in endothelial cells. Vav2 promotes Rac1 activation [GTP loading]. Tiam1 functions as an adaptor protein for VE-cadherin-p67 ^{phox} -par3 polarity complex [36].
Ehop-016, a known inhibitor of Vav2, significantly reduced phagocytosis in normal human trabecular meshwork cells and TM-1 cell line. Knockdown of Rac1 or Tiam1 also inhibited phagocytosis; such effects were reversed by overexpression of constitutively active Rac1 or Tiam1. These studies have also identified ELMO2, RhoG and ILK as upstream regulators of Rac1 in these cells. It is noteworthy that while Ehop-016 inhibited phagocytosis, siRNA-mediated depletion of Vav2 did not exert any significant effects on phagocytosis. Moreover, NSC23766, an inhibitor of Tiam1-Rac1 pathway exerted no clear effects while siRNA-Tiam1 [above] inhibited phagocytosis [37].
Rap1 mediates cell spreading by regulating localization of Tiam1 and Vav2 to sites of active lamellipodia extensions in HeLa cells [38].