Targeting Small GTPases and Their Prenylation in Diabetes Mellitus

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ABSTRACT: A fundamental role of pancreatic β-cells to maintain proper blood glucose level is controlled by the Ras superfamily of small GTPases that undergo post-translational modifications, including prenylation. This covalent attachment with either a farnesyl or a geranylgeranyl group controls their localization, activity, and protein–protein interactions. Small GTPases are critical in maintaining glucose homeostasis acting in the pancreas and metabolically active tissues such as skeletal muscles, liver, or adipocytes. Hyperglycemia-induced upregulation of small GTPases suggests that inhibition of these pathways deserves to be considered as a potential therapeutic approach in treating T2D. This Perspective presents how inhibition of various points in the mevalonate pathway might affect protein prenylation and functioning of diabetes-affected tissues and contribute to chronic inflammation involved in diabetes mellitus (T2D) development. We also demonstrate the currently available molecular tools to decipher the mechanisms linking the mevalonate pathway’s enzymes and GTPases with diabetes.

1. INTRODUCTION

The incidence of diabetes has increased tremendously over the last 50 years, affecting approximately 463 million adults. By 2045, there will be 700 million patients with diabetes. This epidemic is predominantly caused by a rise in the prevalence of type 2 diabetes (T2D), a complex disorder that is characterized by pancreatic β-cell failure with up to 50% cell loss at diagnosis coupled with impaired insulin sensitivity of target tissues, termed insulin resistance (IR). Initially, insulin resistance causes β-cells to secrete more insulin as a way to compensate for the deficiency. Increased metabolic activity of β-cells leads to the formation of reactive oxygen species (ROS) and induction of endoplasmic reticulum (ER) stress that promote inflammation. Initially, a low-grade local inflammation exerts favorable effects, inducing β-cell proliferation and insulin secretion. However, prolonged secretion of inflammatory mediators by β-cells results in proliferation of resident macrophages and recruitment of immune cells from the circulation. Immune cells further contribute to the inflammation that impairs β-cells function and leads to exhaustion.

Enhanced insulin production results in hyperinsulinemia that promotes de novo lipogenesis, hyperlipidemia, and adipose tissue expansion. Expanded adipose tissue supports local and systemic inflammation by enhancing pro-inflammatory mediators secretion, including cytokines, chemokines, and adipokines. Both increased systemic fat and inflammation contribute to the development of IR in the liver and skeletal muscles. Insulin resistance can be observed decades before T2D onset and, together with low-grade chronic inflammation, represents one of the earliest pathogenic events in diabetes-related complications, including cardiovascular disease, diabetic retinopathy, and diabetic kidney disease (DKD) as well as nonalcoholic fatty liver disease (NAFLD). Moreover, insulin resistance, hyperinsulinemia, hyperglycemia, and chronic inflammation are the mechanisms of T2D-associated cancer occurrence and progression. Despite the large panel of treatment options for T2D, including insulin analogues, biguanides, meglitinides, sodium-glucose cotransporter-2 inhibitors, incretin-based therapies, dipeptidyl peptidase 4, α-glucosidase inhibitors, thiazolidinediones, and sulfonylureas, currently available therapies cause side effects and none of them have shown promise in halting the underlying causes of T2D, namely, insulin resistance.

The factors associated with IR, T2D and related comorbidities are complex. However, altered activity and prenylation of small GTPases appears to constitute the link with the pathogenesis. Protein prenylation by isoprenoid groups is a crucial eukaryotic post-translational modification (PTM) of lipids predicted to affect hundreds of proteins in the human proteome. This ubiquitous covalent attachment of farnesyl or geranylgeranyl modulates localization and function of the plethora of signaling proteins. Most prenylated proteins belong to the Ras-related G proteins, particularly Ras, Rab, and Rho that control cell growth, differentiation, proliferation, biomolecule
synthesis, and membrane trafficking. Of interest in this regard, hyperinsulinemia was shown to upregulate prenyltransferases, and selective inhibitors of prenylation markedly increased insulin sensitivity. Moreover, sustained inflammation-induced prenylation of Rho GTPase mediated inhibition of insulin-promoted glucose uptake, causing fasting hyperglycemia.

The isoprenoids used for prenylation are produced by the mevalonate pathway, which is also responsible for cholesterol generation and can be blocked by statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Moreover, statins hamper the production of downstream intermediates, such as FPP (farnesyl pyrophosphate) and GGPP (GRG, geranylgeranyl pyrophosphate, geranylgeranyl diphosphate). However, although statins were reported to improve insulin resistance and reduce systemic inflammation, some studies have shown that statins might have increased the incidence of diabetes. Farnesyl diphosphate synthase (FPPS) and geranylgeranyl diphosphate synthase (GGPPS), downstream of HMG-CoA reductase, catalyze the production of FPP and GGPP, respectively. Biphosphonates (BPs), the inhibitors of FPPS, constitute one of the main classes of drugs used to treat bone-associated diseases. In retrospective cohort studies, the exposure to BPs (alendronate, risedronate) was associated with reduced T2D incidence. Moreover, the administration of BPs was shown to positively affect diabetes-related indices, insulin, fasting plasma glucose (FPG), and hemoglobin A1c (HbA1c).

On the other hand, overexpression of muscle, adipose, and liver GGPPS may contribute to insulin resistance pathogenesis. Therefore, inhibition of FPPS and GGPPS may be considered a strategy for insulin resistance treatment. However, additional large-scale trials are needed to verify these relationships.

The mechanisms by which statins and biphosphonate treatments induce or bypass T2D are not fully understood. It is accepted that their pleiotropic effects might result from changes occurring downstream from these enzymes and that small GTPases are implicated here. Small GTPases are regulated by several protein–protein interactions (PPIs) and PTMs. One of the most studied PTMs is protein prenylation, which is crucial for glucose-stimulated insulin secretion (GSIS) by pancreatic β-cells. However, several proteins within the mevalonate pathway may be implicated in T2D development. Here, we discuss the mechanisms of small GTPase prenylation and how inhibition of various points in the mevalonate pathway might affect protein prenylation and functioning of pancreas and liver, skeletal muscle, kidneys, adipose tissue, and contribute to chronic inflammation involved in T2D development.

2. OVERVIEW OF SUPERFAMILY OF SMALL GTPases AND ENZYMES WITHIN THE MEVALONATE PATHWAY

The human Ras superfamily of small GTPases, including over 150 proteins, comprises five major subfamilies: Ras, Rab, Rho, Ran, and Arf. Six major subgroups (Ras, Rab, Rap, Rad, Rheb, and Rit) have been identified within the Ras subfamily, which includes 36 human members. The Ras branch regulates cell proliferation, differentiation, and survival. With over 60 members in humans, Rab proteins (Ras-related in the brain) form the largest subgroup of the small GTPase superfamily with the principal function of coordinating the transport of proteins and membranes between organelles. Twenty-two genes in humans encode 20 Rho GTPases (Ras homolog) distributed into eight subfamilies (Rac, Cdc42, Rho, RhoD/RhoF, RhoH, RhoU/RhoV, RhoBTB, and Rnd). The Rho family members are essential coordinators of the actin filament network, synchronizing cell shape and movement with intercellular communication, propagation, and differentiation. The single Ran (Ras-related nuclear protein) is one-of-a-kind among other GTPases due to its acidic tail at the C-terminus and the lack of the CAAX motif that precludes attachment to lipid membranes. Ran regulates the transport of molecules between the nucleus and cytoplasm and controls cell cycle progression. The adenosine diphosphate-ribosylation factor (Arf) family comprises 29 members in humans and includes Arf isoforms, Arf-like proteins (Arl), and Sar1 proteins. Arf family lacks the C-terminal prenylation signal. Many of Arf family members are myristoylated at the N-terminus for membrane targeting and control vesicular trafficking, motility, division, apoptosis, and transcriptional regulation.

Small GTPases are guanine nucleotide-dependent molecular switches, active when in complex with GTP and inactive when in complex with GDP. Active small G proteins recruit effectors to the membranes and trigger signal cascades. It requires a tight regulation and small GTPases have three types of controllers, the GTPase-activating proteins (GAPs), the guanine nucleotide exchange factors (GEFs), and the guanine nucleotide dissociation inhibitors (GDIs). GEFs are positive regulators by promoting GDP dissociation, while GAPs are negative regulators by binding to the GTPase and enhancing hydrolysis of GTP. In the case of Rho and Rab, GDIs perturb GAP and GEF regulation and mask the prenyl moiety, thus preventing the association with target membranes (Figure 1A). Abnormal activity of some regulatory proteins is linked to diabetic conditions, e.g., dysregulated production of GD12 contributes to IR.

Members of the small GTPases share a conserved G domain composed of five loops (G1–G5) that are capable of GTP binding and hydrolysis (Figure 1B, in yellow). The G1 motif (P-loop, Figure 1B, in orange) binds the phosphate groups of GTP and GDP, the G2 motif (switch I, Figure 1B, in green) involved in coordinating of Mg²⁺ ion with the β- and γ-phosphate is a site for effector and GAP attachment (Figure 1E: HRas-RasGap). The G3 motif (switch II, Figure 1B, in magenta) activates a catalytic water molecule for GTP to GDP hydrolysis, the G4 motif provides hydrogen bonds with guanine rings, and the G5 region interacts with guanine via water-mediated hydrogen bonds. Upon exchange of GDP to GTP, effector binding is governed by switch I and switch II, very flexible regions, for which the dynamics differ depending on whether GDP or GTP is attached (Figure 1C–E; Supplementary Table 1). The additional C-terminal hypervariable region (HVR), which accommodates a polybasic region (PBR) and cysteines, regulates GTPase association with target membranes (Figure 1B, Supplementary Figure S1).

Small G proteins regulate various effectors (Table 1). GTP binding energy is used to stabilize the switch I and II regions, required for effector recognition (Figure 1C: Rab7a-RILP, 1D: Rac1-Prx1). GTP hydrolysis induces conformational change and a flexibility in the region interacting with the effector. The binding of some effectors slows down GTP hydrolysis, while interaction with GAPs speeds it up.

Besides GDP/GTP binding, small GTPases usually carry a post-translationally attached prenyl tail at cysteine residues present in or located close to the CAAX motif. For that purpose, the farnesyl and geranylgeranyl chains are added to GTPases, and the substrates, FPP and GGPP, are synthesized via the
condensation of the monomers, isopentenyl diphosphate (IPP) with its isomer, dimethylallyl pyrophosphate (DMAPP).\textsuperscript{21}

HMG-CoA reductase produces mevalonate in the rate-limiting step in the pathway. Mammalian HMG-CoA reductase functions as a homotetramer (Figure 3A; Supplementary Table 2). Each monomer consists of the cytosolic C-terminal catalytic domain, the L domain responsible for substrate binding, the S domain binding NADPH, and the N-terminal segment for anchoring to the ER membrane. Statins bind stronger to the L domain than HMG-CoA, e.g., with the inhibitory concentration values of 3.8–6.2 nM for atorvastatin.\textsuperscript{22}

FPPS catalyzes the synthesis of 10-carbon geranyl pyrophosphate (GPP) and the 15-carbon FPP, whereas GGPPS synthesizes the 20-carbon GGPP. Even though free GPP has been detected in cultured human cells,\textsuperscript{23} as far as we know, the geranylated entities have not been detected in human cells yet. The majority of the studies on protein prenylation concentrate on farnesylated and geranylgeranylated proteins and developing the suitable tools.\textsuperscript{24}

Although human FPPS exists as a homodimer (Figure 3B; Supplementary Table 2), human GGPPS is a hexamer assembled from three dimers (Figure 3C; Supplementary Table 2). Despite low sequence identity, both isoprenoid synthases adopt a similar all-α-helical structure. At least three small-molecule binding sites are present in the structure of FPPS, namely, allosteric pocket, allylic substrate (DMAPP and GPP) binding site, and homoallylic substrate (IPP) binding site, with the latter two having high similarity to those found in FPPS. The product inhibitor pocket has been identified in GGPPS as well.\textsuperscript{21}

FPP and GGPP moieties are utilized by four distinct prenyltransferases, namely, farnesyltransferase (FTase), geranylgeranyltransferase I (GGTase-I), Rab geranylgeranyl transferase (GGTase-II/RGGT), and geranylgeranyltransferase III (GGTase-III). All enzymes catalyze the formation of the thioether linkage with the Cys residue located in the prenylation recognition sequence at the C terminus of selected proteins. FTase and GGTase-I transfer a respective prenyl group to protein substrates containing carboxyl-terminal CAAX motifs where C is cysteine, A is aliphatic, and X is any residue. Usually, FTase prefers Cys, Ser, Met, Ala, or Gln while GGTase-I selects Leu, Ile, or Phe at the X position.\textsuperscript{25} Ras, RhoB, and Rheb have been identified as substrates of FTase while GTPases geranylgeranylated by GGTase-I include Rho, Ral, and Rap. There are examples when a protein is either farnesylated or geranylgeranylated, for instance, RhoB. On the other hand, in the case of K-Ras, inhibition of FTase was linked to a compensatory GGTase-I upregulation that can be a reason for the insufficient clinical efficacy of anticancer FTase inhibitors. Therefore, dual FTase/GGTase-I inhibitors may prove a more effective therapeutic approach.\textsuperscript{26}

GGTase-II (Rab geranylgeranyl transferase; RGGT) exclusively geranylgeranylates C-terminally localized CXC and CC motifs in Rab family members. Unlike FTase and GGTase-I, prenylation of Rab proteins by RGGT must be associated with REP1/2 chaperone proteins (Rab escort protein 1/2). Most Rab proteins are doubly geranylgeranylated in a sequential fashion without dissociation of the monoprenyl intermediate.\textsuperscript{25}

The fourth type of protein prenyltransferase, GGTase-III, has been discovered very recently. This enzyme catalyzes the double prenylation of the FBXL2 ubiquitin ligase and Golgi SNARE protein Ykt6 in collaboration with FTase. Chaperone SKP1 protein is required for geranylgeranylation by GGTase-III.\textsuperscript{27,28}

Figure 1. Small GTPase cycle: (A) Interaction with GEF mediates the exchange of GDP for GTP, allows activation, interaction with effectors, and initiation of the signal cascade. Interaction with GAP increases GTP hydrolysis, leading to G protein deactivation. Interaction with GDI keeps small GTPase in an off-state and prevents membrane localization. (B) The conserved architecture of the G domain present in small GTPases (for sequence alignment of Rab, Rho and Ras GTPases implicated in diabetes, see Supplementary Figure S1). (C) Crystal structures of Rab7a: left, inactivated (GDP-bound, PDB: 1VG1); middle, activated (GTP-bound, PDB: 1VG8); right, with its effector RILP (PDB: 1YHN, only part of RILP interacting with Rab7a is shown). (D) Crystal structures of Rac1: left, inactivated (GDP-bound, PDB: 6AGP), middle, activated (GNP-bound, PDB: 3THS); right, with its effector PREx1 (PDB: 4YON, only domains of PREx1 interacting with Rac1 are shown). (E) Crystal structures of HRas: left, inactivated (GDP-bound, PDB: 4Q21); middle, activated (GTP-bound; PDB: 1QRA); right, with RasGAP (PDB: 1WQ1). The P loop is represented in orange, switch I in green, switch II in magenta, coordinated magnesium ion in black, GDP in dark blue, and GTP or GTP analogues in cyan. GNP: phosphoaminophosphonic acid, geranylated ester nonhydrolyzable GTP analogue. The corresponding Supplementary Table 1 contains the list of PDB codes for mammalian small GTPases implicated in diabetes, in GDP and GTP-bound form, with effector/GEF/GAP, when available.

mevalonate pathway (Figure 2). The mevalonate pathway is an essential biosynthetic step that produces components for the cholesterol biosynthesis or FPP and GGPP, and it starts from the

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According to the authors’ knowledge, no inhibitors of this enzyme have been reported yet.

Each prenyltransferase exists as a heterodimer with the active site formed at these proteins’ interface and made up of α- and β-subunits (Figure 3D; Supplementary Table 2). FTase and GGTase-I have different catalytic β-subunits (FTNβ/FTβ and GGT1β, respectively) and share a common α-subunit (FTα). In turn, RGTT and GGTase-III share identical β subunit (RABGGTβ) but contain distinct α subunits (RABGGTα and PTARI, respectively). The RABGGTβ subunit of RGGT and GGTase-III is probably necessary for double prenylation due to its hydrophobic tunnel structure.28

All protein prenyltransferases are metalloenzymes. A Zn2+ ion (a thiolate) is bound by the catalytic domain of the β subunit of GGTases. Additionally, FTase requires Mg2+ that stabilizes PPi leaving group of FPP.
3. SMALL GTPASES AS REGULATORS OF THE INSULIN TRAFFICKING AND EXOCYTOSIS IN PancreATIC β-CELLS

Small GTPases are critical in maintaining whole-body glucose homeostasis acting predominantly in metabolically active tissues, including the pancreas, skeletal muscles, liver and adipocytes. The pancreas plays a key role in this network by secreting the blood-glucose-lowering hormone insulin, produced by β-cells located within islets of Langerhans. Preproinsulin is synthesized on the cytoplasmic side of the ER and translocated to the ER, where the signal peptide is cleaved. The resulting proinsulin is transported to the cis-face of the Golgi apparatus and starts to be packaged after reaching Trans-Golgi Network (TGN). Proteolytic cleavage of proinsulin results in the formation of insulin. Insulin crystallizes with zinc and calcium in the form of dense-core granules during the granule maturation process. The readily releasable pools (RRP) and the reserved pool are two intracellular pools of dense-core insulin granules. When blood glucose level is low, the actin cytoskeleton prevents insulin secretory granules (ISGs) from reaching their release sites. 29 When plasma glucose levels are high in humans, glucose enters the β-cells, primarily through the cell membrane glucose transporters GLUT1 and GLUT3, although GLUT2 expression was also demonstrated by several groups. 30 Upon uptake, glucose is metabolized and a high ATP-to-ADP ratio triggers membrane depolarization by closing ATP-dependent potassium channels (K_{ATP}). Consequently, voltage-gated calcium channels (VGCC) open and that results in calcium influx, which induces docking and fusion with the plasma membrane (exocytosis of insulin granule). The docking and fusion of insulin granules are orchestrated by the soluble N-ethylmaleimide sensitive factor attachment receptor (SNARE) complex. The target-localized (t-SNARE) proteins in the cell surface (SNAP25 and Syntaxin) interact with VAMP (vesicle-associated membrane protein, v-SNARE) on the insulin granules (Figure 4). Under high glucose, the actin cytoskeleton is reorganized, allowing them to move to the plasma membrane. Such glucose-mediated exocytosis of different functional granule pools occurs in response to elevated glucose concentration in a biphasic manner. The rapid first phase (usually the first 10 min) results from fusion and secretion of a subset of plasma membrane-docked granules that are primed with a fully assembled exocytosis machinery (RRP). F-actin filaments are important for the short-range movement of RRP. The second step entails the recruitment of granules from the inside of the cell and microtubule transport. 29

The trafficking of the insulin granules is controlled by several Ras family GTPases and their effectors. Various Rab proteins are associated with the secretory granules and regulate the transport, priming, docking, and fusion of ISGs at the plasma membrane (Figure 3 and Table 1). For example, Rab3 allows ISG docking and tethering at the correct target membrane by interacting with RIM2α and the clustering of the SNARE Syntaxin1 and its binding partner munc18-1. In turn, the Rho family, including Cdc42, Rac, and RhoA, is instrumental in insulin secretion via F-actin remodeling and vesicle fusion regulation. Cdc42 was also shown to be crucial for endocytosis of insulin vesicles. Rap1 and RapA, although less studied, also elicit regulatory effects in insulin release. 19,29 The detailed information on specific functions of small G proteins in insulin secretion by pancreatic β-cells is summarized in Table 1.

Most small GTPases involved in insulin trafficking and secretion are required to be prenylated to function for their biological role and interaction with their respective effectors. FTase, GGTase-I, and GGTase-II are expressed in β-cell lines and pancreatic islets. Studies utilizing inhibitors of HMG-CoA reductase }
reductase (atorvastatin, lovastatin, simvastatin), GGPPS (digeranyl bisphosphonate), FTase (FTI-277, FTI-2628, allyl- or vinyl-farnesols, limonene, manumycin, perlic acid), and GGTase-I (GGTI-298, GGTI-2133, GGTI-2147; GGTI-2368, allyl- or vinyl-geraniols) as well as siRNA-mediated silencing of Rgta and Rggtb revealed that prenylation of small GTPases is essential for β-cell function and insulin secretion.31

4. SMALL GTPASES AS REGULATORS OF GLUT4 TRAFFICKING

Insulin-stimulated glucose uptake into skeletal muscle cells and adipocytes assumes a central role in glucose homeostasis in the body. Most (80–90%) of the infused glucose is absorbed by skeletal muscles that store glucose as glycogen and utilize it in glycolysis; however, adipocytes also exert a critical control in the regulation of blood glucose levels. Insulin promotes the exocytosis of intracellular vesicles containing GLUT4 glucose transporters, the most abundant glucose transporter in muscle and fat cells. In the basal state, GLUT4 locates intracellularly in endosomes, TGN, specialized perinuclear glucose transporter storage vesicles (GSVs), and more peripheral insulin-responsive vesicles (IRVs).63

The insulin binding to the tyrosine kinase receptor activates its autophosphorylation and initiates a signaling cascade starting from phosphorylation of insulin receptor substrates (IRS1 and IRS2). IRS, in turn, phosphorylates phosphatidylinositol-3-kinase (PI3K) and promotes downstream signaling. PI3K constitutes a branch point in insulin signaling activating Akt and Rac1, which in parallel promote GLUT4 transport to the plasma membrane, permitting glucose intake.64 Akt phosphorylates various GAPs (e.g., TBC1D1, TBC1D4), reducing the inactivation of their cognate GTPases (Figure 5). Several Rab GTPases, including Rab4, Rab5, Rab7, Rab8a, Rab10, Rab11, Rab13, Rab14, Rab28, and Rab35, with effector proteins were demonstrated to confer directionality to GLUT4 vesicle traffic. Insulin also activates Rho and Ras GTPases mainly affecting actin remodeling (Table 2). Glucose uptake by GLUT4 also occurs upon muscle contraction; however, muscle contraction and insulin target separate GLUT4 pools. During muscle contraction, the AMP/ATP ratio increases, leading to activation of AMP-activated protein kinase (AMPK), the cellular energy sensor. AMPK, in turn, phosphorylates TBC1D1 and TBC1D4 activating target Rabs.65 Rac1 acts as another contributor to contraction-stimulated glucose transport mediating the stretch-sensitive component.66

5. SMALL GTPASES AND ENZYMES OF THE MEVALONATE PATHWAY IN PATHOLOGICAL STATES OF DIABETES AND ITS COMPLICATIONS

Small GTPases are pivotal in maintaining glucose homeostasis, and aberrant function and regulation of this class of proteins are implicated in the pathological cellular machinery triggered by hyperglycemia. Some reports clearly show glucose-induced upregulation of small GTPases, suggesting that inhibition of such pathways deserves to be considered as a potential therapeutic target in the treatment of T2D and its complications. While expression or activity of Rab members tends to be downregulated under conditions that favor the development of diabetes, overactivated RhoA and Rac1 are involved in many of the pathologies observed in T2D individuals (Table 3). Rac1 is the cytosolic regulatory subunit of the NADPH oxidase (NOX) multicomponent system responsible for ROS generation. Rac1 signaling pathway is implicated in diabetes pathogenesis, mainly by the generation of oxidative stress and islet dysfunction. Hyperactivation of GTP-bound Rac1 is detected in islets derived from T2D patients and animal models.110 Importantly, prenylation of Rac1 might be essential for membrane local-
Rac1 activation is also linked to abnormal retinal neovascularization and ROS production, leading to diabetic retinopathy and vascular dysfunction. In the pancreas, hyperglycemic conditions increase RhoA/ROCK activity that contributes to the diminished GSIS and insulin resistance in muscles. The progression of diabetic kidney disease and vascular complications such as diabetic retinopathy or atherosclerosis have also been connected with elevated levels of RhoA. Taken together, Rac1 and RhoA/ROCK are candidates as new promising targets for pharmacological prevention of islet dysfunction in T2D and T2D-related comorbidities.

GTPase can be targeted directly, through their regulatory proteins or prenylating enzymes. This strategy seems to represent a reasonable approach because increased activity of enzymes within the mevalonate pathway was observed in pathological states of insulin resistance, diabetes, and several T2D-related complications (Table 3).

FPPS expression was elevated in cardiomyocytes and aorta cells from diabetic mice with diabetic cardiomyopathy and atherosclerosis, respectively. FPPS inhibition by alendronate improved fasting plasma glucose, HbA1c, and insulin resistance, lowered the high glucose-stimulated proliferation of VSMCs, and reduced glucose uptake and formation of advanced glycation end products by retinal cells. Notably, in several clinical trials, treatment with bisphosphonates was correlated with a lower risk of T2D (Table 3). In the context of NAFLD, zoledronic acid attenuated hepatic lipid accumulation and improved liver injury by suppressing RhoA activation via decreasing FPP and GGPP farnesyl diphasate levels.

GGPPS inhibition may be another therapeutic strategy in T2D settings characterized by GGPPS overexpression. Although GGPPS was reported to decrease in the islets of T2D patients, this enzyme shows a high expression in the liver, fat and muscles of mice with obesity, IR, and hyperinsulinemia. GGPPS is a crucial mediator linking protein prenylation and metabolic reprogramming, causing NAFLD and subsequent fibrosis development. GGPPS expression was elevated in the livers of mice with obesity-induced hepatic steatosis and NAFLD patients and reduced in hepatocellular carcinoma patients. In adipocytes, chronic exposure to hyperinsulinism makes GGPPS constantly activated. GGPPS further increased prenylation of K-Ras and induced Erk1/2 activation, IRS phosphorylation, contributing to insulin resistance. Knock-down of Ggpps in insulin-resistant adipocytes restored IRS1 phosphorylation and increased insulin sensitivity. Similarly, in mice fed standard chow and high fat diets, knocking out Ggpps in the skeletal muscle increased systemic insulin sensitivity and glucose homeostasis and ameliorated palmitate-induced IR. GGPPS promoted lipid-inflicted IR in skeletal muscles by inducing IRS1 phosphorylation through the geranylgeranylated RhoA/ROCK pathway. Additionally, it was found that ROCK2, and not ROCK1, is involved in the GGPPS-regulated glucose transport in muscle cells, and Rock2 deficiency increases IRS-1/
PI3K/Akt signaling in skeletal muscle and insulin sensitivity in the body. Importantly, any changes in muscle properties in the muscle-specific Ggpps knockout mice were not observed, suggesting that a deficit of GGPP alone probably does not affect muscle morphology and performance. Therefore, GPPPS in skeletal muscle and adipose tissue may be a potential pharmacological target for the prophylaxis of insulin resistance and T2D treatment. This method seems to be more selective for GGTase than FPPS targets, as the second approach decreases cellular FPP, which is used in both prenylation and cholesterol synthesis. As a consequence, a GPPPS targeting drug should have a less off-target effect.

Interestingly, short-term exposure of INS 832/13 β-cells and normal rat islets to an insulinotropic concentration of glucose (20 mM) was shown to stimulate the activities of both FTase and GGTase-I along with increased expression of the α-subunit shared between FTase and GGTase-I. Successively, exposure of INS-1 832/13 cells and normal rodent and human islets to diabetogenic conditions, including long-term exposure to high glucose (30 mM), resulted in a caspase-3-dependent decline in FTase/GGTase-I α-subunit and accumulation of unprenylated Rap1 proteins. These data provide novel mechanistic insights into regulation of FTase and GGTase activities in the β-cells under normal and glucotoxic conditions. Further studies are required to identify factors regulating the expression and activity of pancreatic prenyltransferases under physiological and diabetic conditions. Especially in insulin-sensitive cells (e.g., muscle, liver, and adipose tissue), significant alterations in FTase and GGTases are connected with insulin resistance (Table 3). For example, in skeletal muscles, increased FTase expression and more farnesylated proteins were linked to decreased insulin-stimulated glucose uptake and metabolic changes. FTase inhibitors induce anti-inflammatory effect preventing inducible nitric oxide synthase (iNOS) expression under pathophysiological conditions.

6. STRATEGIES TOWARD REGULATION OF ACTIVITY OF SMALL GTPases VIA THEIR DIRECT TARGETING OR INHIBITION OF MEVALONATE PATHWAY ENZYMES

The involvement of small GTPases and their prenylation in regulating glucose and lipid homeostasis make this class of proteins important in metabolic disorders. Here, we summarize the approaches used to regulate GTPase activity that were reported to be associated with T2D. We concentrate on small molecule modulators that have already been used in diabetes-related studies. Simultaneously, we indicate more recent achievements in the field. The stimulus for widening the range of molecular tools comes from the common use of insufficiently potent inhibitors with not fully validated target(s) and selectivity, which might lead to erroneous results. Therefore, here we highlight the recently introduced compounds of high potency and known selectivity. In many cases, the proposed new molecular tools were applied for cancer...
| GTPase | localization | interacting protein | function | refs |
|--------|--------------|---------------------|----------|-----|
| Rab4a, Rab4b | adipocytes | IRV syntaxin 4 | recycling of GSV via endosomes | Li et al. 67 |
| Rab5a | early endosomes | dynein | insulin signaling deactivates Rab5 and impedes dynein microtubule interaction, slowing GLUT4 inward movement | Chen et al. 68 |
| Rab7 | | | | Ishikura and Klip 96 |
| Rab8a | vesicles in perinuclear region | TBC1D4 (GAP), TBC1D4 (GAP), MyoVb | Rab8A-MyoVb mobilizes GLUT4 vesicles toward the plasma membrane | Sun et al. 71 |
| Rab8a | vesicles in perinuclear region | TBC1D4 (GAP), MyoVb | TBC1D4 in myoblasts and TBC1D1 in myotubes are involved in intracellular retention of GLUT4; Rab8A interacts with MyoVb to translocate GLUT4 | Ishikura and Klip 96 |
| Rab10 | perinuclear endosome/ TGN, GSV | MyoVa | Rab10–MyoVa interaction facilitates the transport of GSVs and docking at the cell surface. | Chen et al. 68 |
| Rab11 | Golgi, endosomes | SEC16A, Exoc6/6b, Exoc7, Rip11 | SEC16A–Rab10 interaction promotes GLUT4 mobilization from the intracellular compartments to the cell to accelerate formation of the GSV | Bruno et al. 74 |
| Rab14 | TGN, endosomes, GSV | TBC1D4 (GAP), TBC1D4 (GAP), TBC1D4 (GAP) | early endosomes-to-TGN transport of GLUT4 | Reed et al. 81 |
| Rab28 | | | | | |
| Rab35 | PM | TBC1D13 (GAP) | GLUT4 translocation (a trafficking pathway from early endosomes) | Davey et al. 83 |
| TC10 | lipid rafts in PM | CIP4/2, N-WASP | GLUT4 trafficking, docking, and fusion with the cell surface | Chang et al. 84 |
| RhoA | PM | ROCK1 | GLUT4 translocation and actin cytoskeleton remodeling | Usui et al. 85 |
| Cdc42 | perinuclear cytosol, PM | P-Rex1 (GEF) | P-Rex1-facilitated GLUT4 plasma membrane association via regulation of the actin cytoskeleton at physiological insulin concentrations | Balamatsias et al. 86 |
| Ras | cytosol, PM | | | | |
| Ra1 | vesicles derived from endosomes, GSV | RGC1/2 (GAP) | mobilization of the exocyst complex to facilitate trafficking of GLUT4 vesicles | Chen et al. 87 |
| Myo1c | | | | | |
| Sec5 and Exo84 | | | | | |
| RaGAP | | | | | |
| Rac1 | | | | | |
| Ra1 | vesicles in perinuclear region | TBC1D15 (GAP) | TBC1D15 is a master regulator of GLUT4 translocation through late endosomal pathway | Wu et al. 88 |
| Rab8a | vesicles in perinuclear region | TBC1D1 (GAP), TBC1D4 (GAP) | TBC1D4 in myoblasts and TBC1D1 in myotubes are involved in intracellular retention of GLUT4; Rab8A interacts with MyoVb to translocate GLUT4 | Ishikura and Klip 96 |
| Rab13 | peripheral vesicles | TBC1D4 (GAP), MICAL-L2 | Rab13 acts at a peripheral step in GLUT4 translocation | Sun et al. 77 |
| Rab14 | vesicles in perinuclear region | TBC1D1 (GAP), TBC1D4 (GAP) | sorting of GLUT4 from the recycling endosome to the insulin-sensitive compartments | Ishikura et al. 99 |
| Rab28 | | | | | |
| | | | | |
related studies, as small GTPases are commonly dysregulated in malignancies, including pancreatic cancer. We believe that their applicability can be extended to other pathological states.

One of the most typical starting points for studies on the mevalonate pathway and GTPases begins with the observation of the effect of statins on diverse cellular processes. Statins target HMG-CoA reductase, the enzyme at the top of the mevalonate pathway. The question arises as to how the observed effect depends on the more downstream elements of the signaling pathway. It can be further investigated by supplying the system with the missing (due to upstream enzyme inhibition) molecules, geranylgeraniol (GGGOH) or farnesol (FOH), or their pyrophosphate analogues GGPP and FPP, respectively. If prenyl alcohols are used, they are converted to the corresponding pyrophosphates in cells and can rescue the effect of the inhibitor. The other solution is to use the inhibitors of more downstream enzymes or compounds interrupting protein–protein interactions to de

Several strategies can be proposed for the control of small GTPases. First, inhibition of the mevalonate pathway’s enzymes, responsible for supplying the farnesyl or geranylgeranyl pyrophosphates, leads to downregulation of small GTPases. Second, a similar result can be expected from the inhibition of enzymes, which use up these pyrophosphates for prenylation of small GTPases. The third approach involves the interruption of regulatory proteins, such as GEFs, GAPs, and GDIs. Fourth, direct targeting of GTPase, e.g., by modulating oncogenic mutant, K-RasG12C, already resulted in the compound investigated in clinical trials. Here, we discuss the above strategies and present selected molecular tools that already have been or can be in the future used in studies which aim at deciphering the diabetes—prenylation mutual dependence.

6.1. Inhibition of HMG-CoA: Statins. The prenylation of small GTPases requires farnesyl and geranylgeranyl pyrophosphates serving as lipid-donating substrates. These are synthesized via the mevalonate pathway. This route is currently targeted by two classes of drugs, statins, inhibitors of HMG-CoA reductase, and bisphosphonates, inhibitors of FPPS. Their pleiotropic effects are the subject of many studies, aimed at determining the extent to which indirect inhibition of downstream enzymes is responsible for these effects.

Statins are the most prescribed drug regimen for treating cardiovascular disease. Their mechanism of action is based on inhibition of HMG-CoA reductase. However, their structural features differentiate them in terms of potency, solubility, and capability to cross the blood–brain barrier. Various studies have been devoted to the role of statins in several diseases, besides their original target, cardiovascular disorders. Their effect was observed in cancer, viral diseases, or parasite infections to name just a few. American Diabetes Association 2019 guidelines recommend the use of statins to T2D patients. Statins have been considered to be anti-inflammatory by inducing the production of anti-inflammatory cytokines which seems to be beneficial for alleviating the systemic inflammation present in diabetic patients. Hyperglycemia promotes inflammation in diabetes by increasing circulating cytokines, activating immune cells, and enhancing their migratory and adhesive capacity. Statin therapy resulted in lower circulating levels of proinflammatory mediators, including C-reactive protein (CRP), IL-1β, IL-6, tumor necrosis factor α (TNF-α), resistin, leptin, visfatin, monococyte chemoattractant protein-1 (MCP-1), intracellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1), and increased concentration of anti-inflammatory adipokine adiponectin. This human pro-monocytic cell line cultured in high glucose and stimulated with LPS showed reduced release of TNF-α, IL-1β, IL-6, and MMP1 after statin treatment. Inhibition of MMP1 expression by statins was achieved through targeting protein prenylation-mediated ERK activation and could be partially rescued by GGPP. The effect was due to Ras and Rac prenylation as the addition of GGTagase-I inhibitor exerted a similar effect to statins. Moreover, statins...
### Table 3. Diabetes-Related Alterations in Ras GTPases and Associated Enzymes of Mevalonate Pathway

| GTPase | abnormality | refs |
|--------|-------------|------|
| **β-cells** | | |
| **Ras GTPases** | | |
| Rab1a | Rab1a expression is down-regulated in islets of Goto-Kakizaki rats with T2D | Liu et al. 32 |
| Rab2a | under chronic high glucose, Rab2A effector GAPDH undergoes poly(ADP-ribose)ylation and dissociation that impairs Rab2A activity | Sugawara et al. 33 |
| Rab3a | Decreased Rab3a expression under exposure to conditions that promote the development of T2D (proinflammatory cytokines, fatty acids, or oxidized low-density lipoproteins) | Ljubicic et al. 47 |
| Rab7 | Rab7-dependent upregulated RILP expression in diabetic rats or mice causes a reduction of ISGs and promotes proinsulin degradation | Zhou et al. 59 |
| Rab27a | decreased Rab27a expression upon exposure to conditions mimicking T2D | Abderrahmani et al. 129 |
| Rab37 | decreased Rab37 expression under exposure to conditions that promote the development of T2D (proinflammatory cytokines, fatty acids, or oxidized low-density lipoproteins) | Ljubicic et al. 47 |
| RhoA | hyperglycemic conditions increase RhoA/ROCK activity that enhances the growth of stress fibers and diminishes GSIS | Kong et al. 114 |
| RhoA | RhoA mRNAs levels are higher under lipotoxic conditions in INS cells | Malnigren et al. 130 |
| Rac1 | Rac1 prenylation is indispensable for glucose-stimulated NOX2 activation and ROS production | Syed et al. 131 |
| Rac1 | Rac1 is translocated to the membrane under hyperglycemia, hyperlipidemia and increased ROS production | Zhou et al. 132 |
| Tiam1 and prenylation-dependent Rac1 activation is pivotal for cytokine-stimulated NOX2 activation and ROS production | Veluthakal et al. 133 |
| hyperglycemic conditions increase association between β-PiX (GEF) and Rac1 | | |
| Tiam1: Rac1-NOX2 signaling mediates impaired mitochondrial function in the β-cell in response to increased glucose, lipids, or pro-inflammatory cytokines; prenylation of Rac1 is crucial for its membrane translocation and activation of NOX2 | Kowlu et al. 134 |
| boosts PP2A-Rac1-mediated signaling in metabolic stress-caused β-cell dysfunction | Elumalai et al. 135 |
| Rac1: NOX2 signaling pathway induces CD36 trafficking to the cell surface and amplifies influx of free fatty acids resulting in the dysfunction of β-cells | | |
| **enzymes of the mevalonate pathway** | | |
| FTase/ GGTase-I | high glucose stimulates the expression of the common α-subunit of FTase/GGTase-I without affecting β-subunits and increases the activities of FTase and GGTase-I | Goalstone et al. 126 |
| guco- and lipotoxic ER stress conditions activate caspase-3-mediated cleavage of the α-subunit of FTase and GGTase-I, leading to their inactivation | Veluthakal et al. 127 |
| adipocytes | | |
| Rab4a, Rab4b | Rab4a and Rab4b mRNA and protein levels are reduced in epidydimal fat in obese diabetic db/db mice; Rab4b mRNA expression is decreased in subcutaneous fat in pathologically obese patients with diabetes | Kada et al. 130 |
| Rab5a | Rab5a mRNA expression is increased in subcutaneous fat in pathologically obese diabetic patients | Kada et al. 130 |
| Rab11a | Rab11a mRNA expression is increased in subcutaneous fat in pathologically obese diabetic patients | Kada et al. 130 |
| Rab18 | the presence of Rab18 in human adipose tissue is correlated to obesity; Rab18 overexpression participates in hydrolysis of triacylglycerols | Pulido et al. 139 |
| dysregulated production of lumican and GDI2 contributes to IR in obese individuals through modification of collagen I organization and alters lipid storage by inhibiting binding of Rab18 to lipid droplets | Guzmán-Ruiz et al. 20 |
| RND3 | RND3 mRNA is elevated in obesity and associates positively with insulin resistance; RND3-mediated stimulation of lipolysis leads to insulin resistance; RND3 is farnesylated but it has no intrinsic GTPase activity (insensitive to GAPs) | Dankel et al. 140 |
| Ras | GGPPS-induced Ras prenylation leads to chronic Erk1/2 signaling in hyperinsulinemia | Shen et al. 141 |
| **enzymes of the mevalonate pathway** | | |
| GGPPS | Elevated GGPPS expression in insulin-resistant adipose tissues of db/db mice | Vicent et al. 14 |
| hyperinsulinemia stimulates GGPPS and K-Ras by increasing geranylation; Rab/MAPK/Erk1/2 signaling leads to IRS-1 phosphorylation and insulin resistance; knock-down of Ggpps in insulin-resistant adipocytes restores insulin sensitivity | Shen et al. 15 |
| FTase | hyperinsulinemia promotes the phosphorylation of the α-subunit of FTase and potentiates activation of p21Ras by growth factors | Goalstone et al. 141 |
| **skeletal muscle** | | |
| Ras GTPases | | |
| Rab1A | Rab1a is upregulated in skeletal muscles of HFD-fed mice and in mitochondria of skeletal muscle from T2D patients | Chae et al. 143 |
| RND3 | RND3 is elevated in skeletal muscle of T2D patients defective ROCK1 activity due to increased RND3 expression is connected with insulin resistance in skeletal muscles of obese T2D humans; in mice, ROCK1 deficiency causes whole-body IR as well as defects in insulin signaling in skeletal muscle | Chun et al. 144 |
| RhoA | RhoA/ROCK signaling under obese and insulin-resistant conditions strains insulin pathway via phosphorylation of IRS-1 | Kanda et al. 115 |
| RhoA | upregulation of mitochondrial RhoA in T2D patients | Chae et al. 143 |
| Rad | Rad mRNA is increased in muscles of T2D individuals; Rad lacks typical prenylation motifs resulting in a primary cytosolic location | Reynet et al. 145 |
| Rad | Rad overexpression inhibits glucose transport in muscle cells | Coletta et al. 146 |
| Rad | | Moyers et al. 147 |
Table 3. continued

| GTPase | abnormality | refs |
|--------|-------------|------|
| FTase  | Reduced insulin-stimulated glucose uptake in muscle is related with augmented FTase expression and more farnesylated proteins | Nakazawa et al. 128 |
| Rab24  | Rab24 is upregulated in the livers of obese NAFLD patients and positively correlates with increased body fat content. Rab24 inhibition in the liver improves autophagic flux and mitochondrial connectivity, resulting in a reduction in hepatic steatosis | Seitz et al. 149 |
| GGPPS  | GGPPS fosters lipid-induced IR in muscle by activating the RhoA/ROCK signaling; GGPPS is overexpressed in skeletal muscles of ob/ob mice | Vicent et al. 14 |
| GFPPS  | GFPPS is highly expressed in the livers of NAFLD patients; mice with liver-specific GGPPS knockout are protected from HFD-inflicted hepatic steatosis | Liu et al. 160 |
| FTase  | Reduced insulin-stimulated glucose uptake in muscle is related with augmented FTase expression and more farnesylated proteins | Nakazawa et al. 128 |
| Rab24  | Rab24 is upregulated in the livers of obese NAFLD patients and positively correlates with increased body fat content. Rab24 inhibition in the liver improves autophagic flux and mitochondrial connectivity, resulting in a reduction in hepatic steatosis | Seitz et al. 149 |
| GGPPS  | GGPPS fosters lipid-induced IR in muscle by activating the RhoA/ROCK signaling; GGPPS is overexpressed in skeletal muscles of ob/ob mice | Vicent et al. 14 |
| GFPPS  | GFPPS is highly expressed in the livers of NAFLD patients; mice with liver-specific GGPPS knockout are protected from HFD-inflicted hepatic steatosis | Liu et al. 160 |
| FTase  | Reduced insulin-stimulated glucose uptake in muscle is related with augmented FTase expression and more farnesylated proteins | Nakazawa et al. 128 |

Ras GTPases

- **GGPPS**
  -GGPPS fosters lipid-induced IR in muscle by activating the RhoA/ROCK signaling; GGPPS is overexpressed in skeletal muscles of ob/ob mice
  -GGPPS-controlled prenylation mediates lipid-induced insulin resistance by augmenting RhoA/ROCK signaling.
  -ROCK2, but not ROCK1, mediates the GGPPS-regulated PI3K/Akt pathway and glucose transport

- **FTase**
  -Reduced insulin-stimulated glucose uptake in muscle is related with augmented FTase expression and more farnesylated proteins

**Liver and non-alcoholic fatty liver disease (NAFLD)**

- **Rab24**
  -Rab24 is upregulated in the livers of obese NAFLD patients and positively correlates with increased body fat content. Rab24 inhibition in the liver improves autophagic flux and mitochondrial connectivity, resulting in a reduction in hepatic steatosis

**Diabetic kidney disease (DKD)**

- **RhoA**
  -RhoA is increased in human mesangial cells induced by hyperglycemia and subsequently Rho/ROCK signaling
  -RhoA/ROCK signaling plays a role in the pathogenesis of diabetic kidney disease through glomerular sclerosis signaling pathways and extracellular matrix deposition
  -RhoA translocation to cell membrane is increased in diabetic renal cortex

**Diabetic retinopathy**

- **Ras**
  -High glucose increases the growth of VSMCs (vascular smooth muscle cells) and c-fos gene expression through Ras/ROCK
  -High glucose stimulates VSMC proliferation through Ras-Raf-ERK1/2 pathway responsible for atherosclerosis progression
  -H-Ras and its effector, Raf-1, are increased in diabetic retinopathy; prenylation of Ras is essential for glucose-mediated effects in the retina in diabetes

**diabetes-accelerated macrovascular complications**

- **HMG-CoA reductase**
  -High glucose induces HMG-CoA reductase overexpression in aortas from diabetics and cultured VSMCs
  -Hyperglycemic conditions result in Rac1 and endothelial dysfunction with abnormal platelet function.

- **FFPPS**
  -High glucose induces FFPPS overexpression in aortas from diabetics and cultured VSMCs

- **GGPPS**
  -High glucose induces GGPPS overexpression in aortas from diabetics and cultured VSMCs

- **FTase**
  -High glucose induces FTase overexpression in aortas from diabetics and cultured VSMCs

- **GGTase-I**
  -High glucose induces GGTase-I overexpression in aortas from diabetics and cultured VSMCs

Lowered resistin expression in 3T3-L1 adipocytes, human preadipocytes and monocytes/macrophages. Immune cells from diabetic patients who underwent statin therapy showed lower expression of activation markers, lymphocyte function-associated antigen-1 (LFA-1), very late activation antigen-4 (VLA-4), and CD18, and decreased activation potential. Pravastatin and fluvastatin decreased the adherence of neutrophils and monocytes to human endothelial cells under high glucose conditions by reducing the surface expression of endothelial adhesion molecules (intercellular adhesion molecule-1 (ICAM-1), P-selectin, and E-selectin). Further, statin treatment inhibited NF-κBp65 and MAPK proinflammatory signaling pathways in monocytes from T1D patients, muscle cells from streptozotocin (STZ)-treated rats, and aortic endothelial cells cultured under high glucose. The effect was H-Ras-mediated, as dominant-negative H-Ras (S17N) exerted an effect similar to that with statin treatment. Atorvastatin and rosuvastatin improved antigen-specific immunity and cytotoxic activity of T cells in diabetic mice. However, statins were also demonstrated to contribute to the proinflammatory environments in diabetes. Statins can activate
inhibitors of FTase induced a caspase-3-mediated decline in the levels of prenylated proteins, such as nuclear lamins, leading to β-cell dysregulation and death.\textsuperscript{199} High-dose statin treatment slowed the progression of coronary atherosclerosis, resulting in disease regression in both diabetic and nondiabetic patients.\textsuperscript{200} Although several questions remain unanswered, statins increase T2D risk, with some statins showing a stronger association (e.g., simvastatin, rosuvastatin, and atorvastatin) than others (e.g., pravastatin).\textsuperscript{11} Additionally, as the generation of mevalonate derivatives is blocked by statins and the former regulates the expression of HMG-CoA reductase via multiple feedback mechanisms, there is an observed remarkable increase in HMG-CoA levels. This restricts the effectiveness of the drug and instigates more intensive treatments that may lead to side effects.\textsuperscript{200} Thus, treatment of insulin resistance, T2D, and T2D-related complications with HMG-CoA reductase inhibitors may be a viable option.

6.2. Inhibition of FPPS: Bisphosphonates and Non-phosphorus Analogues. The most potent inhibitors of FPPS and GGPPS belong to the bisphosphonates, chemically stable analogues of pyrophosphates, the natural substrates of these enzymes. Bisphosphonate inhibitors of FPPS constitute a known drug class. They bind to hydroxyapatite in bone tissue because of the Ca\textsuperscript{2+} chelating properties of the α,α-bisphosphonic acid motif. They show high selectivity for osteoclasts deposited in bone minerals, and therefore, they are used to restrain osteoclast-mediated bone resorption. Bisphosphonates are also used in patients with cancers causing osteolysis, and some studies show their antitumor activity. However, the charged nature of this group makes them challenging to employ for other therapeutic applications, due to high bone affinity and low serum levels in nonbone applications, low cell membrane permeability, and high clearance by the kidneys.\textsuperscript{211} Still, a number of reports have shown that administration of bisphosphonates could be associated with a reduction in the risk of incident T2D,\textsuperscript{12} reduced glucose uptake, formation of glycation end products, insulin resistance,\textsuperscript{212} and hepatic lipid accumulation.\textsuperscript{212} These effects were observed in various tissues affected by diabetes, including the retina and liver (Table 5).

Nitrogen-containing bisphosphonates (N-BP), such as zoledronic acid, risendronic acid, alendronic acid, pamidronic acid, and minodronic acid, belong to the clinically validated inhibitors of FPPS (Table 5 and 6). They compete for binding in the allicy site of FPPS with the natural substrates, DMAPP and GPP. The search for inhibitors of human FPPS binding at the active site did not bring nanomolar potency inhibitors without bisphosphonic moiety. Therefore, attempts were directed at identifying inhibitors targeting the allosteric site near the C-terminus of the enzyme.\textsuperscript{217} Several such nonbisphosphonate classes of inhibitors were proposed,\textsuperscript{207–210} e.g., 1–4, although not all of them bind inside the FPPS allosteric pocket.\textsuperscript{210} Although these compounds were designed to have superior “druglike” properties in comparison to the bisphosphonates, none of them showed notable antitumor activity in cell-based tests. To the best of our knowledge, their potential in diabetes-related studies has not been investigated yet. That is why here we show only selected examples, limiting cases to those tested for human FPPS and showing nanomolar potency (Table 6).

6.3. Inhibition of GGPPS: Lipophilic Bisphosphonates. The enzyme responsible for the synthesis of geranylgeranyl pyrophosphate is GGPPS, and it is now intensively studied as a potential drug target.\textsuperscript{221}
Table 4. Selected Statins and Their Application as Tools to Study Diabetes and Inflammation

| Statin     | Diabetes- and inflammation-related studies                                                                                                                                                                                                 | Reference            |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| simvastatin| Inhibits the activation of Ras promoted by high glucose and down-regulates tube-like formations in the co-culture of mesangial cells with HUVEC; alleviates urinary albumin secretion and VEGF protein expression in the kidneys of diabetic rats (diabetic nephropathy) | Ho et al.202          |
|            | Diminishes GLUT2 expression via a reduction of ATP production in pancreatic β-cells                                                                                                                                                           | Zhou et al.203       |
|            | Attenuates thermal hyperalgesia and mechanical allodynia in diabetic mice and relieves the symptoms of painful diabetic neuropathies                                                                                                                                                  | Ohnawa et al.204     |
|            | Simvastatin or atorvastatin treatment result in the reduction in serum CRP and IL-6 in patients with abnormal glucose hemostasis                                                                                                                                              | Milajerdi et al.182  |
|            | Reduces serum MCP-1 in T2D patients                                                                                                                                                                                                           | Dworacka et al.177   |
|            | Reduces levels of TNF-α, IL-6 and visfatin in gingival crevicular fluid of T2D patients with chronic periodontitis                                                                                                                             | Bahammam et al.176   |
|            | Decreases plasma CRP, CD40 ligand and IL-8 in T1D patients; reduces levels of monocyte superoxide anion, IL-8, TNF-α and NFKb in LPS-activated monocytes from T1D patients                                                                 | Jialal et al.174     |
|            | Reduces up-regulation of serum MCP-1 and ICAM-1 in STZ-induced diabetes rats                                                                                                                                                                  | Lin et al.181        |
|            | Reduces levels of leukocyte activation markers (LFA-1, VLA-4 and CD18) and CD14 receptor on monocytes from T2D patients                                                                                                                                                 | Stule et al.186      |
|            | Decreases production of cytokines in PHA-stimulated lymphocytes (IL-2 and IFN-γ and TNF-α) and LPS-stimulated monocytes (TNF-α, IL-1β, IL-6, MCP-1) from patients with T2D and mixed dyslipidemia                                                          | Krystiak et al.183   |
|            | Inhibits MMP-1 expression by targeting Ras and Rac prenylation and ERK1/2 activation in LPS-stimulated U937 human pro-monocytic cell line                                                                                                                                 | Sundararaj et al.184 |
| atorvastatin| Atorvastatin and pravastatin inhibit GLUT2 expression, however, rosuvastatin and pitavastatin show a slight increase in GLUT2 expression in β-cells                                                                                                                                 | Zhao & Zhou193       |
|            | Decreases insulin-stimulated 2-deoxyglucose uptake in 3T3L1 adipocytes linked to blocking of GLUT4 translocation into the plasma membrane; reduces the active membrane fraction (prenylated) of both RhoA and Rab1                                                                 | Takaguri et al.187   |
|            | Reduces insulin synthesis in β-cells by inhibiting the activation of the Ras complex pathway                                                                                                                                                  | Sun et al.188        |
|            | Reduces serum resistin levels in T2D and resistin expression in 3T3-L1 adipocytes cell line, human preadipocytes and monocytes/macrophages                                                                                                                         | Ichida et al.175     |
|            | Lowers serum levels of IL-6 and TNF-α in T2D patients                                                                                                                                                                                       | Usharani et al.178   |
|            | Reduced serum CRP in patients with abnormal glucose hemostasis after atorvastatin or simvastatin treatment, and decreased serum IL-6 levels after atorvastatin treatment                                                                                                     | Milajerdi et al.182  |
|            | Decreases plasma resistin and leptin in T2D patients                                                                                                                                                                                       | von Eynatten et al.179|
|            | Decreases serum visfatin in T2D patients                                                                                                                                                                                                       | Kadoglou et al.195   |
|            | Increases serum adiponectin in T2D patients with high risk of cardiovascular disease                                                                                                                                                           | Soran et al.186      |
|            | Lowers serum levels of MCP-1 and VCAM-1 in T2D patients                                                                                                                                                                                    | Dworacka et al.177   |
|            | Reduces NAD(P)H activity, expression of NF-kBp65, VCAM-1, TNF-α and ILβ and phosphorylation of ERK1/2 in STZ-treated rats                                                                                                                             | Riad et al.189       |
|            | Reduces ICAM1 expression on endothelial cells, and monocyte adhesion under high glucose condition                                                                                                                                             | Park et al.188       |
|            | Induces NLRP3/caspase-1 inflammasome activation and IL-1β-dependent IR in adipose tissue                                                                                                                                                         | Henrikhsbo et al.193 |
|            | Atorvastatin and pravastatin inhibit GLUT2 expression, however, rosuvastatin and pitavastatin show a slight increase in GLUT2 expression in β-cells                                                                                                                                 | Zhao & Zhou197       |
The elevated expression of GGPPS was induced by high glucose levels. Its high abundance was observed in a number of tissues of obese and/or diabetic patients, promoting, for example, lipid-induced muscle insulin resistance. However, up to now, the GGPPS inhibitors were not used in diabetes-related studies. Instead, inhibitors of upstream enzymes in the mevalonate pathway were applied or the experiments were run on cells with GGPPS knock-down. Therefore, here we show that direct inhibitors of GGPPS do exist and we present the selective and the most potent among them as available chemical tools to study diabetes-related processes.

The number of selective GGPPS inhibitors is limited, partially due to the previously held conviction that dual FPPS and GGPPS inhibitors are more efficient as antitumor agents. Despite the low sequence identity between human FFPS and GGPPS (17%), their tertiary (but not quaternary) structures are surprisingly similar and their catalytic mechanisms are probably similar. Many attempts at obtaining GGPPS inhibitors led to the development of dual FPPS and GGPPS inhibitors, such as compound 8 (Figure 7), which is about 100 times more potent than zoledronic acid in obstructing tumor growth, or compound 7, which represents another chemo-

---

**Table 5. Selected Inhibitors of FPPS**

| Compound          | Potency   | Diabetes-related activity                                                                 | References       |
|-------------------|-----------|------------------------------------------------------------------------------------------|------------------|
| Alendronate       | IC₅₀=460 nM | Protective effect for incident diabetes                                                    | Chen et al.      |
|                   |           | Improves fasting plasma glucose, HbA1c and insulin resistance in postmenopausal women    | Fard et al.      |
|                   |           | Positive effect on glucose control in elderly osteoporotic women with senile diabetes    | Maggeri et al.   |
|                   |           | Reduced glucose uptake and formation of advanced glycation end products in pretreated   | Lee et al.       |
|                   |           | retinal cells at high glucose condition (10 μM)                                          |                  |
|                   |           | Attenuates diabetic atherosclerosis development (15 mg/kg/day; 16 weeks; intragastric    | Chen et al.      |
|                   |           | route) and high glucose-induced proliferation of VSMCs (30 μM, 100 μM)                   |                  |
| Pamidronate       | IC₅₀=500 nM | Reduced glucose uptake and formation of advanced glycation end products in pretreated   | Lee et al.       |
|                   |           | retinal cells at high glucose condition (10 μM)                                          |                  |
| Zoledronate       | IC₅₀=4.1 nM | Attenuates hepatic lipid accumulation and improves liver injury through suppressing RhoA activation via decreasing FPP and GGPPS levels (50 μg/kg or 200 μg/kg, every 2 days for 30 days; IV) | Tang et al.      |
|                   |           | Suppression of VSMCs proliferation (10 μM)                                               | Wu et al.        |

"Proinflammatory cytokines: IL-1β, IL-2, IL-6, TNF-α. Proinflammatory chemokines: IL-8, MCP-1. Proinflammatory adipokines: leptin, resistin, visfatin. Anti-inflammatory adipokines: adiponectin. Adhesion molecules: ICAM-1, VCAM-1, E-selectin, P-selectin. Proteases: MMP-1. Signaling pathways: ERK, NF-κB."
type of GGPPS bisphosphonate inhibitors and shows ∼15× higher activity toward GGPPS, compared with FPPS.\textsuperscript{223} The FPPS inhibitors are usually smaller molecules, having a shorter alkyl chain and a positive-charge feature. The GGPPS bisphosphonate inhibitors contain one or two large hydrophobic groups, they lack hydroxyl group in C-α, and there is no positive charge required. Therefore, they are more lipophilic, which makes them more prone to targeting nonbone tissues.\textsuperscript{207} The broadest class of GGPPS inhibitors contains a bisphosphonic acid moiety, which is a substitute of the unstable pyrophosphate residue. It turned out that digeranylated bisphosphonic acid 5, representing the so-called V-shaped molecules, shows 0.2 μM activity against GGPPS and no inhibition of farnesylation.\textsuperscript{221,224} At least one geranyl or longer isoprenoid chain is required for inhibition of GGPPS; these prenyl chains occupy the substrate and product binding sites, FPP and GGPP, respectively.\textsuperscript{225} Several such V-shaped compounds\textsuperscript{224,226} including those that contain an ether bond, 6,\textsuperscript{226} and the so-called U-shaped analogues were prepared.\textsuperscript{227} Recent works show the anticancer therapeutic potential of several hydrophobic bisphosphonates. However, the most interesting group is constituted by triazoles\textsuperscript{228} that carry an isoprenoid chain (Figure 7). The homogeranyl and homoneryl triazole analogues, 9, turned out to be the most potent GGPPS inhibitors reported, demonstrating high selectivity in inhibiting GGPPS vs FPPS. They can slow pancreatic tumor growth in vivo.\textsuperscript{229} The preliminary studies on metabolic stability and pharmacokinetics indicate that they are metabolically stable in human liver microsomes.\textsuperscript{230} Most analogues showed a higher potency of the Z isomer. An interesting property was observed for 9, as studies demonstrated that the two isomers interact synergistically, making the mixture more potent than a single isomer. It is tentatively explained as resulting from synergistic binding in both the substrate, FPP, and product, GGPP,
inhibitory channels.\(^2\) In the case of analogues bearing a methyl group at C-\(\alpha\), compound 10, the activity against GGPPS was similar for both isomers, 0.086 mM for (\(Z\))-10 and 0.125 mM for (\(E\))-10.\(^3\) Additionally, such a design, with the locked C-\(\alpha\), enables the prodrug form preparation to overcome the bioavailability hurdles of bisphosphonic drugs.\(^3\)

### 6.4. Inhibition of Prenylating Enzyme, FTase, and Direct Targeting of Ras Proteins

Ras proteins regulate cell proliferation, differentiation, and survival. The most known members of the Ras subfamily are Harvey-Ras (H-Ras), neuroblastoma-Ras (N-Ras), and Kirsten-Ras (K-Ras). K-Ras is the most commonly mutated protein in many cancers, accounting for almost 85% of all Ras mutations.\(^4\) The K-Ras\(^{12D}\) mutation is the most prevalent in pancreatic and colorectal cancers. G12 is located at the protein active site, interacting with a phosphate-binding loop (P-loop) and two switch regions, which control binding to effector and regulatory proteins. The oncogenic K-Ras mutation inhibits GTP hydrolysis (by weakening its GTPase activity or hampering the GAP-stimulated GTP hydrolysis), making such mutants constantly active and activating downstream effectors.\(^3\)

In the early efforts to control the activity of Ras, the inhibition of FTase was the most widely developed approach. FTase is responsible for PTMs of Ras, enabling their proper localization in the membrane, often after additional modifications, such as palmitoylation. While several FTIs (FTase inhibitors) were developed, they failed in clinical trials due to alternative prenylation with GGTase-I, which restored their membrane association. There is renewed interest in FTase inhibitors, as their efficacy against the regulation of H-Ras activity has been verified. Out of a few dozen trials, one FTI small molecule drug, lonafarnib (commercially available from Sigma-Aldrich), has...
been recently approved by the U.S. Food and Drug Administration [FDA; https://www.fda.gov/drugs/drug-approvals-and-databases/drug-trials-snapshots-zokinvy] for the therapy of Hutchinson-Gilford Progeria Syndrome and certain progeroid laminopathies. Several other drug candidates are at various stages of preclinical or clinical trials to prevent or treat cancer, such as manumycin-A, FTI-277, tipifarnib, L778123, and BMS-214662.170

Several other strategies directly targeting Ras proteins have been developed. Besides the use of biologics, such as monoclonal antibodies, mimetics of antibody variable fragments, and antisense oligonucleotides, efforts have been undertaken to interrupt the association between Ras and regulatory or effector proteins, such as phosphodiesterase-δ, Sos, Raf, or Tiam1. A breakthrough strategy has been developed for selective targeting of a mutant variant of K-RasG12C and small molecules, such as AMGS10, MRTX849, ARS3248, and LY3499446 covalently modifying the mutant cysteine, that has progressed to clinical trials (e.g., NCT04380753, NCT04667234). 235 Recently, Crews and collaborators have shown the potential of a PROTAC molecule, LC-2, developed from the covalent K-RasG12C inhibitor (MRTX849) linked with the VHL (von Hippel-Lindau ligase) ligand, which turned out to be an efficient K-Ras degrader. 248 Several reviews have been recently published covering these topics [see refs 232 and 235].

Few studies were devoted to selective targeting of another mutant K-RasG12D, the most prevalent in pancreatic cancer. Sakamoto et al. introduced K-RasG12D KS-58, derived from KReppe-2d (Ac-RRRRCPLYISYDPVCRRRR-NH2), which inhibited interactions with two proteins, RasGDP-Sos1 (GDP-GTP exchange) and RasGDP-BRaf. It inhibits both GDP- and GTP-bound K-RasG12D. Despite its molecular weight (1333.6 g/mol) and negatively charged polar residue, it showed anticancer activity in vivo, making it a potential lead compound. 234

To the best of our knowledge, Ras proteins have not been directly associated with diabetes yet, as their misregulation is more connected with cancer. However, several reports indicate that hyperglycemia and/or hyperinsulinemia stimulate the expression and/or activation of FTase (Table 3). Therefore, we listed some FTase inhibitors (Table 7), concentrating on those that have been already used in diabetes-related studies or are at various stages in clinical trials. Most of them are commercially available, which makes them accessible for many laboratories. On the other hand, the repurposing strategy for already studied (potential) therapeutics has many advantages. Such agents have already undergone thorough examinations in terms of their toxicity, bioavailability, and other aspects, which need consideration in drug development. For more information on the plethora of FTase inhibitors, please refer to recent reviews [see refs 232 and 235].

### 6.5. Inhibition of Prenylating Enzymes: GGTase-I

GGTase-I inhibitors have received less attention than inhibitors of FTase. GGT-I inhibitors often serve in combination with FTIs in order to inhibit prenylation and function of oncogenesis.

| Compound | Potency (target) | Compound | Potency (target) |
|----------|-----------------|----------|-----------------|
| BMS-214662 | ICSIC=3.35 nM (FTase) | Liu et al.245 |
|          | 6 completed clinical trials, Phase 1 (e.g. ClinicalTrials.gov Identifier: NCT0006213) | 39 clinical trials (e.g. ClinicalTrials.gov Identifier: NCT0773474) |
| tipifarnib | ICSIC=0.86 nM (FTase) | 87 clinical trials (e.g. recruiting, Phase 2 ClinicalTrials.gov Identifier: NCT0248774) | |
|          | KS-58 is derived from KReppe-2d, whose Kd=50 nM for K-RasG12D (K-RasG12D) Sakamoto et al. 234 | Almost complete loss of association of Rho with RhoGDI; enzyme assay: GGTase-I ICSIC=8.24 nM; FTase ICSIC>2 μM; | |
|          | ICSIC= 2 nM (FTase); ICSIC= 98 nM (GGTase-I) Lobell et al. 248 | FTase ICSIC=1.4 nM; GGTase-I ICSIC=1.7 μM | Sun et al. 246 |
| FTCI276 combined with cationic guanidyl-containing moiety disrupts electrostatic driven acidic interfaces of FTase and GGTase-I: Tsukamoto et al. 249 | 11a: Ki = 0.0006 μM (FTase); Ki = 0.71 (GGTase-I) | | |

| Table 8. Selective and Dual Inhibitors of FTase and GGTase-I and Direct Inhibitor of K-Ras that Have Potential to Be Used in Diabetes-Related Studies |
Table 9. Compounds Interrupting the Protein–Protein Interactions of Rho GTPases Applied in Diabetes-Related Research (Part A) and Those That Have Potential to Be Used in Future Diabetes-Related Studies (Part B)

| Part A | Compound | Target Potency | Diabetes-related activity | Reference |
|--------|----------|----------------|--------------------------|-----------|
|        | Blocks Rac1-Tiam1 and Rac1-TiroN interactions 50-100 μM under the GST pull-down conditions and in NIH 3T3 cells del Mar Maldonado & Dhamawardhana203 | Reduces hyperglycemia-stimulated p38MAPK signal in isolated β-cells | Sidrala et al.207 | |
|        |          |                | Prevents diabetes in the NOD mice; suppresses ER stress in NOD islets; 2.5mg/kg (IP) | Velthuika I et al.153 | |
|        |          |                | Protection from hyperglycemia-induced endothelial dysfunction, restoring NO levels, and reducing oxidative stress; antplatelet effect (reducing platelet aggregation) during hyperglycemic conditions: 30 μM; 5 mg/kg (IP) | Schiat- tarella et al.191 | |
|        |          |                | Protection from acceleration of capillary cell apoptosis and mitochondrial damage (diabetic retinopathy); in the retina diabetic model it protects from diabetes-induced increase in ROS; 2.5 mg/kg (IP); 20 μM | Kowlu et al.154 | |
|        | Inhibits Rac1 activity (IC50=1 μM in MDA-MB-435 cells; inhibitor of Cdc42 at ≥10 μM) blocks Rac1-Vav2 interaction del Mar Maldonado & Dhamawardhana203 | Reduces high hyperglycemia-activated p38MAPK signaling in isolated β-cells; 5 μM | Sidrala et al.207 | |
|        |          |                | Inhibition of glucose-induced activation of Rac1-NOx2–ROS signaling in retinal endothelial cells; amelioration of the development of retinopathy and functional/structural abnormalities mitochondrial damage in diabetic mice; 5 μM; 25 mg/kg (IP) | Moham- mad et al.156 | |
|        | Nucleotide (GTP) binding inhibitor for Rac1; IC50=10-50 μM del Mar Maldonado & Dhamawardhana203 | Reduces high glucose-initiated p38MAPK cascade in isolated β-cells; 10 mM | Sidrala et al.207 | |
|        |          |                | Inhibition of platelet aggregation in diabetic conditions (at a higher dose of 100 μM) | Schiat- tarella et al.191 | |
|        |          |                | Inhibition of mast cell degranulation, 40 μM | Sheshacha lam et al.206 | |
|        | Inhibits RhoA-GEF interaction including LARG, DBL, LBC, p115 RhoGEF or PDZ RhoGEF (30 μM) Shang et al.261 | Inhibition of mast cell degranulation but less and differently than EHT 1864 40 μM | Sheshacha lam et al.206 | |
|        | Nonspe- cific ROCK inhibitor (Ki=0.33 μM for ROCK1, IC50 of 0.158 μM, 4.58 μM, 12.30 μM, 1.650 μM for ROCK2 and PKA, PKC, PKG, respectively Chen et al.262 | glucose and lipid metabolism is corrected in obese Zucker rats, with correction of serine phosphorylation in IRS-1 and insulin signaling in skeletal muscles | Kanda et al.156 | |
|        |          |                | Prevents the development and progression of hyperglycemia, dyslipidemias, obesity and nephropathy in diabetic OLETF rats | Kikuchi et al.263 | |
|        |          |                | Ameliorates the endothelial dysfunction in STZ-induced diabetic rats through reducing the TNF-α-mediated NADPH oxidase activation | Hofmi et al.264 | |
|        |          |                | A renoprotective agent for the treatment of diabetic nephropathy | Gu et al.265 | |
|        |          |                | Attenuates high glucose-induced monocyte adhesion to endothelial cells; through limiting expression of endothelial VCAM-1 and monocyte MCP-1 (preventing diabetes associated vascular inflammation and atherogenesis) | Li et al.266 | |
|        | ROCK inhibitor IC50 = 122 nM (ROCK1) IC50 = 52 nM (ROCK2) AS1892802 | AS1892802 is a novel and attractive analgesic agent which may be useful in treating diabetic neuropathy | Yoshimi et al.267 | |
|        | Selective ROCK inhibitor K1= 220 nM (ROCK1) K2= 300 nM (ROCK2) Y-27632 | Y-27632 and fasudil reverse the high glucose-induced expression of TNF-α and reduce glomerular fibrosis and inflammation (diabetic kidney disease) | Chen et al.156 | |
|        |          |                | Increases secretion of GLP1 and glucose tolerance | Petersen et al.268 | |
|        |          |                | Attenuates thermal hyperalgesia and mechanical allodynia in diabetic mice (the symptoms of painful diabetic neuropathies) | Ohnawa et al.269 | |
drivers, K-Ras and N-Ras proteins. Blocking only FTase activity led to alternative prenylation of FTase substrates by GGTase-I. Therefore, several dual inhibitors of these two prenyl transferases were also developed.

Interestingly, this research area also evolved in a different direction: the development of agents directly targeting the GGTase-I substrates, Rho GTPases. This gives an alternative pathway for the selective regulation of particular GTPases. This topic is covered in the following paragraph.

Although GGTase-I is an attractive target for cancer-related studies, its inhibitors are rarely used in diabetes research. GGTase-I might be overexpressed under high glucose concentrations (Table 3), while its knock-down blocked diabetes-accelerated atherosclerosis, which might be related to interfering with Rac1 geranylgeranylation, finally inhibiting ROS production, and ERK1/2 and JNK signaling.

Peptidomimetics of the CAAX motif in protein substrate and dihydropyrrole or tetrahydropyridine-based analogues constitute two main classes of GGTase-I inhibitors. Here, we listed inhibitors of GGTase-I, giving priority to molecules that have already been used in diabetes-related studies. Among them, we find selective a GGTase-I inhibitor, GGTI-2147, and FGTI-2734, which show dual inhibition of FTase and GGTase-I. The representative of dihydropyrrole analogues, P61-A6, was applied in the design of targeted delivery of P61-A6 to pancreatic cancer cells. For that purpose, the GGTase-I inhibitor (or in combination with FTase inhibitor) was encapsulated into liposomes, which upon exposure to the lower pH of cancerous cells was released.

There are some representatives of GGT-I inhibitors, which have potential in future studies as they are of nanomolar potency, are commercially available and commonly applied in biological studies, or show different degrees of selectivity against FTase vs GGTase-I. We also include GGTI-2418 as the only GGTase-I inhibitor currently in clinical trials. Selected examples of such compounds are listed in the Tables 7 and 8.

6.6. Direct Targeting of Rho GTPases. The strategy based on inhibition of GGTase-I alone or in combination with FTase is limited by its nonselectivity in terms of affecting many GTPases. The efforts to directly and selectively target Rho GTPase ended with success. The most studied representatives of Rho GTPases are Rac1, RhoA, and Cdc42, which are often overexpressed in malignancies, as they are regulators of cancer cell migration and invasion. The subfamilies of Rho GTPases interact with each
other and are controlled by regulatory proteins and effectors. Their hyperactivation can result from their mutations, down-regulation of GAPs, or upregulation of GEFs. The latter interaction is the most commonly targeted. As the topic of regulation of Rho GTPases has been widely summarized recently, here we concentrate on selected inhibitors, directly targeting Rac1 and RhoA, as the connections of these with diabetes-related malfunctions are the most broadly reported (Table 9).

As has been already mentioned, one of the most popular strategies to inhibit Rac1 activation is the interruption of its binding with GEFs. There are several Rac1-Tiam1 (GEF) (T-cell lymphoma invasion and metastasis 1) inhibitors. The structural studies identified the specific amino acid residues. In addition to small molecule inhibitors, there were attempts to develop peptide-derived Rac1-Tiam1 inhibitors.

In the case of RhoA regulation, it was found that GGPPS promotes lipid-induced insulin resistance in muscle by enhancing RhoA/ROCK signaling. It could be prevented by inhibition of GGPPS or RhoA/ROCK interaction. Several ROCK kinase inhibitors have been developed and used as tools in diabetes-related studies (Table 9). However, one needs to remember that the ROCK pathway is essential for many cellular processes and Rac and Cdc42 are crucial regulators of a plethora of cell signaling receptors. Therefore, more selective approaches are needed.

In Table 9, we present inhibitors that can potentially be used as probes, as they interrupt protein–protein interactions that are important in diabetes. Among them, we can distinguish inhibitors of Rac1 interaction with GEFs such as P-Rex1, Vav2, or Trio. Another mechanism works for compound 12 and 13 that by blocking interaction with nucleotide disrupts binding between Rac1 and PAK1.

6.7. Inhibition of Prenylating Enzymes: GGTase-II. The abnormal activities of GGTase-II and some Rab proteins have been identified in several diseases, including cancer, such as pancreas, breast, skin, colon, lung, ovarian, and prostate, to name just a few. GGTase-II alone was not reported to be up- or downregulated in diabetes, but some Rab GTPases can be associated with various aspects of T2D (Table 3). Up to now, in most identified cases, the pathological effect of dysregulation of Rab GTPases was associated with their impaired activity. However, in a few cases, Rab GTPase was upregulated, e.g., Rab24 in the livers of obese NAFLD patients correlated with body fat content. Since the current state of knowledge implies that, in diabetes, the upregulation of Rabs is required to reverse the pathological state, new strategies need to be developed. Here, we discuss the approaches that have been studied to date to present the currently available tools.

Several attempts have been made to control GTPases; however, these approaches are not very diversified. One of the most studied strategies is based on the development of inhibitors of GGTase-II. This enzyme was proven to be a druggable target. Several classes of small molecule inhibitors have been developed (compounds representing these classes (15–24) are presented in Figure 8), differing in their mode of action (e.g., inhibitors of first or second geranylgeranylation), selectivity (versus other prenyltransferases), and potency. GGTase-II inhibition is limited by the lack of substrate selectivity, as it affects all or most Rab GTPases. The most active analogues contain a tetrahydrobenzodiazepine motif (compound 15). Only in the case of α-phosphonocarboxylates (19–23), the selectivity toward different Rabs was reported. This class of inhibitors prohibits the introduction only of the second geranylgeranyl group to Rabs, leaving the monogeranylated Rabs unaffected. Among the currently known phosphonocar-
Another strategy is based on the direct targeting of Rab GTPases. Only few such attempts have been reported in the literature. These studies involved analysis of the protein–protein interaction surfaces in order to design molecules mimicking them. These studies resulted in the development of stapled peptides, STRIP16, which targets Rab8a, mimicking its interaction with RIP.286 and RFP14, blocking Rab25:FIP complex formation, in which FIP is the effector protein.287 Although these studies were also dedicated to optimizing the stability and bioavailability of these inhibitors, they need further refinement.

7. Recent Strategies for Selective Targeting of Inhibitors to Diabetes-Affected Organs

The small GTPases and their regulatory proteins are omnipresent in all kinds of cells. Therefore, when planning to use the inhibitors in diabetes-related studies, specific delivery to certain tissues needs to be considered to increase their efficiency and bioavailability while reducing toxicity and dosing frequency. A number of reviews exist that describe organ-specific delivery systems288 and produg strategies, including those that show a possible masking of ionic phosphonic groups, with the latter being so popular among the compounds described in this Perspective.289 Here we selected several approaches targeting tissues related with diabetes.

The development of various types of antidiabetic drugs has been accompanied by the constant progress in the field of their delivery, especially in terms of the effective and convenient transport of insulin, a protein, which due to its unstable nature cannot be delivered orally. Peptide-derived therapeutics have limited oral bioavailability due to their destruction by gastric acid and proteolytic enzymes and the limited absorption from the intestine. However, medicinal chemistry has developed several strategies to overcome these hurdles, based on various structural modifications (e.g., PEGylation, attachment of cell-penetrating peptides) or coapplication of enzyme inhibitors. That topic has been broadly described in many medicinal chemistry textbooks. In the case of peptides and other classes of therapeutics, the transportation and targeting can be improved by the use of nanocarrier delivery systems, which include liposomes, niosomes, polymeric nanoparticles or micelles, and dendrimers.290 When the drug is encapsulated within a nanostructure, such a nanomaterial presents both opportunities, such as the possibility of surface modification with a tissue-targeting moiety as well as safety concerns, variable efficiency, outcome of biomaterial degradation, and possible side effects. The field of nanodelivery is under constant development, and one needs to be aware that such studies require additional caution, but the potential of nanocarriers cannot be denied. Here, we present examples of the recently reported strategies or reviews for selectively targeting drugs to β-cells, liver cells, adipocytes, and muscle cells.

The interesting feature of β-cells is an exceptionally high concentration of zinc ions (up to ~30 mM) while the zinc concentration in the cytosol in most cells is ~400 pM.291 Zn(II) can catalyze hydrolytic reactions, which can be used to ignite the activity of the released cargo. Because of the above features, many attempts were reported to design a system for imaging β-cells.292

That feature was used for attaching a zinc-chelating residue onto a β-cell replication-inducing compound.293 Another study involved designing a prodrug consisting of an inactivated drug linked with a Zn(II)-binding ligand. Such an approach was applied for the targeted release of fluorochromes and β-cell mitogenic compouds in human β-cells.292 In both cases, the hybrid compounds preferentially accumulated within β-cells. Upon reaching the Zn(II)-abundant environment, the bond between the cargo and the Zn(II)-binding scaffold was cleaved, releasing the active cargo.

In the last 20 years, diverse strategies have been developed for noninvasive imaging of β-cells for diagnostics. For that purpose, a number of β-cell-surface-specific proteins, often overexpressed, were used, such as vesicular monoamine transporter 2 (VMAT2), sulphonylurea receptor (SUR-1), glucagon-like peptide 1 (GLP-1), free fatty acid receptor 1 (FFAR1), and β-cell-specific antigens. Some of the markers used for β-cell imaging can be used to design targeting molecules, such as monoclonal antibodies, to selectively deliver a drug, which will be cleaved upon reaching the target.294 To recognize the surface-specific protein, antibody–drug conjugates could be used, which recently have gained importance as an attractive approach for cell-specific targeting. Although challenging, GPCR-specific monoclonal antibodies are also being developed, and the first ones, erenumab and mogamulizumab, were recently approved by the FDA.295

These strategies were developed for certain tissues affected by nondiabetes-related pathological states, such as cancer, liver fibrosis, and muscle aging. Analogous strategies can be applied for the targeted delivery of drugs to the tissues affected by diabetes. Still, careful evaluation needs to be conducted to determine to what extent the developed methods can be applied for diabetes-stricken organs.

For selective targeting to the liver, several delivery methods, including the ones that use surface markers, were developed for liver cancer cells296 and proposed for liver fibrosis.297 In the case of muscle cells and adipocytes, selective targeting is challenging because of their high representation in the body. However, for skeletal muscle, surface recognition elements were identified and used for selective uptake. In addition to small molecules like carnitine (a drug linked with carnitine shows improved muscle uptake via OCTN2 transport), monoclonal antibodies, or viral vectors,298 aptamers have also been proposed as a muscle-specific delivery vehicle.299

8. Future Perspective

The involvement of small GTPases and their prenylation in regulating glucose and lipid homeostasis makes this class of proteins important in metabolic disorders. Inhibitors of protein prenylation have been investigated as potential therapeutics to treat multiple diseases. Statins, used primarily as cholesterol-lowering drugs, were also found to reduce systemic inflammatory responses independently of cholesterol. Various clinical trials demonstrated that treatment with statins decreased soluble proinflammatory mediators and lowered the activation capacity of monocytes and lymphocytes.176,177,179,182,208 In vitro studies identified statin targets as being small GTPases (Ras, Rac and Rho).174,184,190 On the other hand, accumulating evidence suggests that statins enhance the inflammatory responses and elevate the risk of diabetes.11 The evidence for statin-mediated effects points toward the NLRP3 inflammasome/caspase-1 complex, and this could be a new target in the treatment of inflammation in diabetes.192,193 However, there may be more still-unexplored prenylation targets that contribute to increased inflammation upon exposure to statins. Thus, decreasing the
activity of enzymes that are downstream from HMG-CoA reductase in the mevalonate pathway may be a promising strategy for treating insulin resistance and diabetes. Pro- and anti-inflammatory effects of statins could be explained by the opposite outcomes of the mevalonate pathway’s inhibition, depending on the tissue, euglycemia versus hyperglycemia, and target type. Enhancing prenylation may localize specific GTPases and thus enhance its function. It may also sequester it away from its effectors and reduce the effect. Further studies should be conducted to assess how prenylation controls inflammation and insulin sensitivity in muscle, liver, and adipose tissue, and insulin production and secretion by pancreatic islets. Statins, inhibitors of other enzymes in the mevalonate pathway, as well as GTPase activation inhibitors should be employed to identify the specific factors that enhance or reduce inflammation and contribute to insulin resistant β-cell dysfunction. It will further our knowledge about the function of prenylation in diabetes and allow the development of more context-specific treatments.

Defective or upregulated prenylation can contribute to the decrease of metabolic cell viability and dysfunction in pancreatic β-cells. Several enzymes are decreased in the islets of T2D patients while they are upregulated in the liver, adipose tissue, and muscles in individuals with obesity, insulin resistance, and hyperinsulinemia (Table 3). Therefore, further studies are required to identify factors regulating the expression and activity of pancreatic prenyltransferases under physiological and diabetic conditions. More work needs to be done to show which signaling pathway is essential for desired efficacy. Moreover, a better understanding of how the beneficial effect from preclinical T2D models can be effectively translated to T2D patients is needed.

After a broad search for the interconnections between small GTPases and different proteins and processes in T2D, we summarized the approaches that can be used to regulate GTPases activity in pathological cellular machinery triggered by hyperglycemia. We concentrated on small molecules. It is crucial to be cautious when using inhibitors, both those newly reported as well as such that are known for some time. The proper molecular probe should be potent and selective toward the validated molecular target. Otherwise, such studies might repeatedly generate uncertain or even erroneous results. Therefore, here, besides showing the previously used chemical probes, sometimes not of the highest quality, we highlight the recently introduced compounds of high potency and known selectivity.

We described the most common strategies used to control small GTPases, via inhibition of the mevalonate pathway and prenylating enzymes, or the interactions between GTPases and their regulatory proteins, such as GEFs. In the case of most GTPases, there has been significant progress in developing chemical tools—potent and selective inhibitors—allowing further studies. However, most approaches studied involve the downregulation of GTPases, while expression or activity of Rab GTPases tends to be downregulated under conditions that favor the development of diabetes. In addition to targeting the gene expression, no other strategy to achieve Rab upregulation has been applied yet. Here, the opportunity might be spotted at targeting the interactions with regulatory proteins, such as GAP and GDI, which bind Rabs and inactivates them under normal circumstances. Also, downstream effectors, or other post-translational modifications, such as phosphorylation/dephosphorylation, ubiquitination, palmitoylation, and serotonylation, can be targeted.

In diabetes-related studies, the apparent targets among GAPs constitute TBC1D1 and TBC1D4, which are Akt targets in insulin-stimulated GLUT4 traffic. Mutations in TBC1D1 and TBC1D4 are linked with obesity and insulin resistance in humans. Phosphorylation of TBC1D1 and TBC1D4 is thought to shut down their GAP function, leading to increased levels of active Rab GTPases, which triggers GLUT4 translocation.

However, these different approaches are not straightforward. Individual functions of the different Rab proteins that undergo various post-translational modifications, such as phosphorylation, serotonylation, AMPylation, phosphocholinlation, palmitoylation, and ubiquitination, often occur at localization, which affects the interaction with diverse proteins GAPs, GDIs, and effectors. Only a few such interactions have been already identified, and only in a few cases it was determined when the interaction with the effector is taking place, after or before particular post-translational modification. Phosphorylation of Rabs is still poorly recognized in terms of its role, mechanistic implications, and regulation via kinase-phosphatase-mediated modifications. The different sites might be phosphorylated by different kinases, leading to diverse effects and distinct distribution of Rabs, altering the activity of GAPs, GEFs, effectors, and others. Also, phosphorylation of Rab GTPases may be reversible through the action of protein phosphatases, which may reverse the signaling cascade. The four locations of phosphorylation were recently distinguished. For example, the phosphorylation at switch II may interfere with Rab–GAP interaction, simultaneously increasing or decreasing the interaction with the effector protein. On the other hand, phosphorylation within the α3/β5 loop antagonizes the catalytic activity of another kinase, LRRK2.

It is the future task to comprehend how small GTPases are linked to diabetes and related disorders. In addition to the application of existing small molecular tools, continuously developing technologies, such as (phospho)proteome- and genome-wide screening, could be used as a measure to identify the various partners of small GTPases, including their mutual dependencies.

**ASSOCIATED CONTENT**

* Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00410.

List of crystal structures of small GTPases playing a role in diabetes, corresponding to Figure 1; list of crystal structures of the enzymes of mevalonate pathway playing a role in diabetes mellitus, corresponding to Figure 3; amino acid sequence alignment of human GTPases involved in diabetes and insulin resistance (PDF)

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Notes
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Abbreviations
Akt, protein kinase B; Arp2/3, actin-related protein 2/3 complex; BP, bisphosphonate; CD, cluster of differentiation; CRP, C-reactive protein; DKD, diabetic kidney disease; DMAPP, dimethylallyl pyrophosphate; ER, endoplasmic reticulum; ERGIC, ER-Golgi intermediate compartment; ERK, extracellular-signal-regulated kinase; FOH, farnesol; FPP, farnesyl pyrophosphate; FPPS, farnesyl pyrophosphate synthase; FTase, farnesyltransferase; GPP, geranyl pyrophosphate; FTI, FTase inhibitor; GPP, geranylgeraniol; GPP (or GRG), geranylgeranyl pyrophosphate, geranylgeranyl diphosphate; GPPPS, geranylgeranyl pyrophosphate synthase; GGTase-I, geranylgeranylation transferase type I; GGTase-II, Rab geranylgeranyltransferase; GGTase-III, geranylgeranylationtransferase III; GLUT, glucose transporter; GSIS, glucose-stimulated insulin secretion; GSV, GLUT4 storage vesicles; HbA1c, hemoglobin A1c; HMG-CoA, 3-hydroxymethyl-3-methylglutaryl coenzyme A; ICAM-1, intracellular adhesion molecule 1; IgG, immunoglobulin G; IL, interleukin; IR, insulin resistance; ISG, insulin secretory granule; IPP, isopentenyl diphosphate; IR, insulin, resistance; IRS, insulin receptor substrate; IRV, insulin-responsive vesicles; Kir2, inwardly rectifying potassium channel 2; LFA-1, lymphocyte function-associated antigen; LPS, lipopolysaccharides; MCP-1, monocyte chemotactic protein-1; MMP-1, matrix metalloproteinase-1; NAPFDL, nonalcoholic fatty liver disease; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NOX, NADPH oxidase; p65, LNR3NOD-like receptor family pyrin domain containing 3 inflammasome; PAK1, P21-activated kinase 1; PDK, phosphoinositide-dependent kinase; PHA, phytohemagglutinin; PM, plasma membrane; RGGT, Rab geranylgeranyl transferase; ROS, reactive oxygen species; RRP, readily releasable pool; SNARE, soluble N-ethylmaleimide sensitive factor attachment receptor; STZ, streptozotocin; SUR1, sulfonylurea receptor-1, a regulatory subunit of ATP-sensitive potassium channel; TCA cycle, tricarboxylic acid cycle; T2D, type 2 diabetes; TGN, Trans-Golgi Network; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; VGCC, voltage-gated calcium channel; VSMC, vascular smooth muscle cell

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