MOLECULAR
METABOLISM

GPRC6A: Jack of all metabolism (or master of none)

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ABSTRACT

Background: GPRC6A, a widely expressed G-protein coupled receptor, is proposed to be a master regulator of complex endocrine networks and metabolic processes. GPRC6A is activated by multiple ligands, including osteocalcin (Ocn), testosterone (T), basic amino acids, and various cations.
Scope of Review: We review the controversy surrounding GPRC6A functions. In mice, GPRC6A is proposed to integrate metabolic functions through the coordinated secretion of hormones, including insulin, GLP-1, T, and IL-6, and direct effects of this receptor to control glucose and fat metabolism in the liver, skeletal muscle, and fat. Loss-of-GPRC6A results in metabolic syndrome (MetS), and activation of GPRC6A stimulates proliferation of β-cells, increases peripheral insulin sensitivity, and protects against high fat diet (HFD) induced metabolic abnormalities in most mouse models. Bone, cardiovascular, immune, and skin functions of GPRC6A have also been identified in mice. Expression of GPRC6A is increased in prostate cancer (PCa) cells, and inhibition of GPRC6A attenuates PCa progression in mouse models. The function of GPRC6A in humans, however, is not clear. During evolution, a unique polymorphism of GPRC6A emerged mainly in humans of Asian and European decent that has been proposed to alter membrane trafficking and function. In contrast, the ancestral allele found in all other species is retained in 1%, 15%, and 40% of people of Asian, European and African descent, respectively, suggesting GPRC6A gene variants may contribute to the racial disparities in the risk of developing MetS and PCa.
Major Conclusions: If the regulatory functions of GPRC6A identified in mice translate to humans, and polymorphisms in GPRC6A are found to predict racial disparities in human diseases, GPRC6A may be a new gene target to predict, prevent, and treat MetS, PCa, and other disorders impacted by GPRC6A.

1. INTRODUCTION

A new understanding of energy homeostasis initially proposed that osteocalcin (Ocn), a bone-derived hormone, is released into the circulation in response to insulin-mediated bone resorption, and activates GPRC6A, a member of the Family C, G-protein coupled receptors, to stimulate insulin and testosterone secretion in β- and Leydig-cells, respectively [1−4]. The existence of this novel Ocn-GPRC6A endocrine network regulating energy metabolism and sex hormone production was supported by mouse genetic studies [5] [6]. In this regard, both Ocn$^{-/-}$ and Gprc6a$^{-/-}$ mice exhibit impaired glucose tolerance, insulin resistance, obesity, hepatosteatosis, and low circulating testosterone (T) [7−9]; compound double heterozygous GPRCA$^{+/-}$ and Ocn$^{+/-}$ mice show additive effects on these metabolic abnormalities [10], and conditional deletion of GPRC6A in pancreatic β-cells [10,11] and testicular Leydig cells [2,12] prevents Ocn stimulation of insulin and T production, respectively.
Based on an expanded understanding of GPRC6A expression and functions, an updated schema proposes a more complex network, where GPRC6A is activated by multiple ligands, and has broader functions in many more tissues than originally envisioned [14]. In addition to the ligand Ocn, GPRC6A mediates the rapid, non-genomic signaling responses to T and is also activated by basic amino acids and cations. GPRC6A is expressed in liver hepatocytes, skeletal muscle myocytes, and possibly adipocytes, in which direct effects of this receptor to regulate glucose and fat metabolism have been described. Activation of GPRC6A stimulates the production and secretion of key, metabolically active hormones in addition to insulin from islets [7,8,11] and T from Leydig cells [2,7]. GPRC6A also stimulates release of glucagon-like peptide-1 (GLP-1) from intestinal cells [15−17], adiponectin from adipocytes [18,19], and interleukin 6 (IL-6) from myocytes [20,21] to create novel inter-organ communications (Figure 1). GPRC6A is expressed in the prostate, upregulated in prostate cancer cells and tumors, and implicated in prostate cancer (PCa) risk and progression GPRC6A may provide a molecular basis for the well-known association between metabolic syndrome (MetS) and the risk of PCa [22] (Figure 1).

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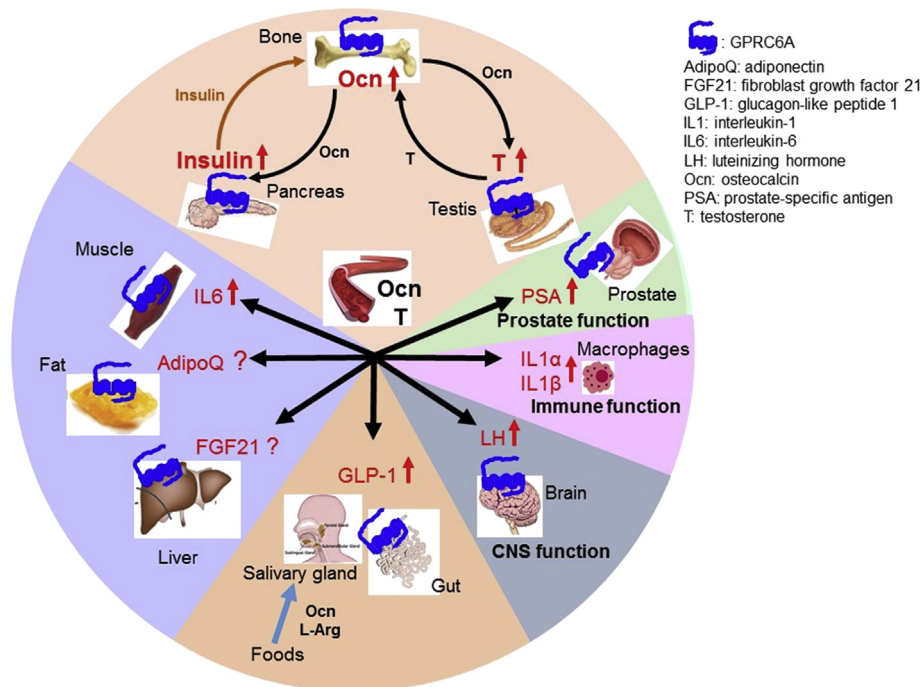
Figure 1: GPRC6A and its ligands create complex networks regulating energy metabolism GPRC6A is expressed in multiple tissues and activated by osteocalcin (Ocn), testosterone (T), basic amino acids, L-Arg, and divalent and trivalent cations. GPRC6A regulates energy metabolism through direct effects on β-cells to stimulate proliferation and insulin secretion, and on muscle, fat, and liver to regulate IL-6, adipokines, and hepatokines, as well as glucose and fat metabolism. GPRC6A in salivary glands and small intestines responds to amino acids and results in increased secretion of GLP-1. GPRC6A is expressed in the brain, where it regulates LH secretion and myelin content. GPRC6A also is expressed in macrophages and modulates innate immune responses and cytokine release in response to cations. GPRC6A is also expressed in the prostate gland and is upregulated in prostate cancer. GPRC6A is expressed in bone, where it regulates bone mineralization, and in skin where it regulates cell proliferation.

More importantly, evolutionary divergence of GPRC6A in humans suggests that this receptor has undergone important changes in its biological functions. Modern humans, Neanderthals, and Denisovans (all genus *Homo*) have a common polymorphism of GPRC6A that substitutes a "K..Y" amino acid sequence at position 744 in the third 3rd intracellular loop for a highly evolutionarily conserved RKLP sequence present in all other vertebrates (Figure 2A). In the 1000 human genomes project, the uniquely human "K..Y" allele is present in 99.4% of the East Asian descent population, 91.8% of the European descent population, 83.4% of the American Hispanic population, and 59.9% of the persons of African descent (Figure 2B). In contrast, the ancestral "RKLP" sequence in 3rd intracellular loop of GPRC6A, which is evolutionarily conserved in all pre-human species, is present only in a minority of humans, and is considered a variant (rs386705086) that is most commonly found in African-derived populations (Figure 2C). The emergence of the "K..Y" polymorphism only in hominids and its preservation during evolution indicate a selection pressure that modifies the physiological function of this allele. On the other hand, the persistence of the ancestral RKLP sequence and its predominant expression in humans of African descent (the oldest humans) suggest that this allele may contribute to the racial disparities in diseases associated with GPRC6A, including MetS [23], and PCa [24].

1.1. Knowledge gaps and controversies

This schema is controversial in part, because it is difficult to conceive of a physiological purpose for a single receptor to sense so many disparate ligands and influence numerous metabolic hormones and organ-specific metabolic processes. Some remain committed to the original simple view of Ocn activation of GPRC6A and do not attempt to incorporate either the effects of basic amino acids and testosterone to

activate this receptor or the broad effects of GPRC6A to regulate diverse tissue functions beyond tissue involved in energy metabolism [1]. Others posit that only basic amino acids, and neither Ocn nor T, play physiological roles to activate GPRC6A, a nihilist view that GPRC6A has no function in humans [25,26]. Thus, progress to date places GPRC6A on the precipice of either being a master regulator of metabolic processes or, alternatively, on the list of loss-of-function tolerant gene variants that have little clinical impact in humans [27]. Which of these suppositions is correct will determine if GPRC6A participates in the pathogenesis of human diseases and is a valid target to develop agonists and antagonists to respectively treat MetS and PCa.

Though there are several issues that need to be resolved, the majority of evidence points to an important role for GPRC6A in mice, and a likely role of GPRC6A in modulating the risks of high-fat-diet induced MetS, and racial disparities in the risk of MetS and PCa in humans. These premises are supported by the following observations.

1.2. Molecular basis for multiple ligand activation of GPRC6A (Figure 3)

As noted, GPRC6A is activated by multiple ligands, including cations, such as calcium, zinc, and magnesium, the basic amino acids L-arginine (L-Arg), L-lysine (L-Lys), and L-ornithine (L-Orn), the bone-derived peptide Ocn, and T. GPRC6A mediates the non-genomic effects of testosterone [28]. Are all of these physiologically relevant ligands for GPRC6A, and, if so, what is the structural basis for such ligand diversity, and how do these work together or separately to regulate GPRC6A tissue functions?

There is a general consensus that L-Arg activates GPRC6A *in vitro*, but the ability of L-Arg administration to regulate metabolic process through activation of GPRC6A *in vivo* has produced conflicting results
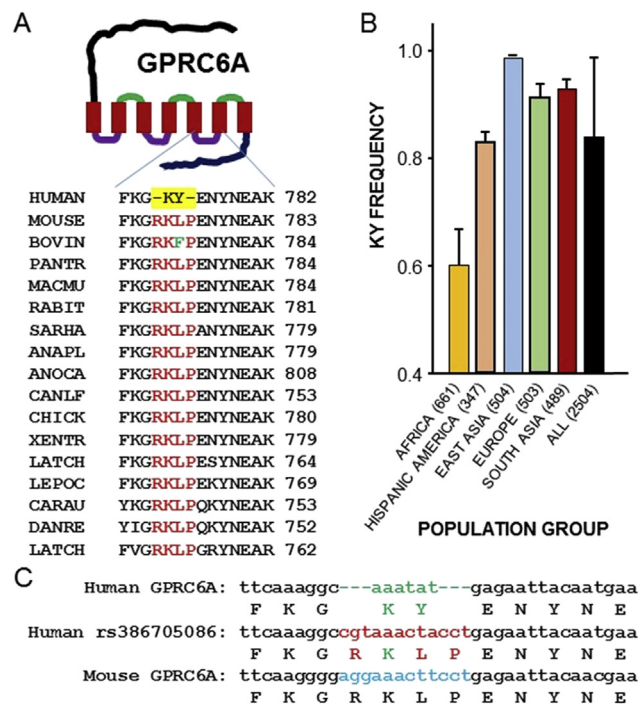
Figure 2: Evolutionarily divergence of GPRC6A in humans. A) Comparison of the amino acid sequence of the 3rd intracellular loop of GPRC6A from different species, including human (HUMAN), mouse (MOUSE), bovine (BOVIN), chimpanzee (PANTR), rhesus macaque (MACMU), rabbit (RABIT), tasmanian devil (SARHA), mallard (ANAPL), green anole lizard (ANOCA), dog (CANLF), chicken (CHICK), *Xenopus tropicalis* (XENTR), coelacanth (LATCH), spotted gar (LEPOC), zebra finch (TAEGU), goldfish (CARAU), and zebrafish (DANRE). Only humans have the K..Y substitution for the ancestral RKLP sequence. B) Distribution of the KY and RKLP alleles in humans grouped by geographical area. Each population group contains a minimum of 4 and maximum of 7 subpopulations. Data are from www.1000genomes.org. C) Comparison of nucleotides and amino acid sequence of the 3rd intracellular loop of GPRC6A from human GPRC6A and mouse. The K..Y allele is the human referent sequence, and the RKLP ancestral sequence in mouse GPRC6A is considered to be the rs386705086 polymorphism in humans.

[9,29]. L-Arg, derived from dietary intake or protein catabolism, might be expected to regulate metabolic functions through GPRC6A, but to date a link between GPRC6A and metabolic effects of protein intake have not been established [30]. There is an intriguing and yet-to-be validated possibility that GPRC6A may function intracellularly to sense lysosomal amino acids (*vide infra*). To date, there have been no demonstrable *in vivo* divalent cation sensing functions of GPRC6A, however, the trivalent cation aluminum, which activates GPRC6A *in vitro*, have been shown to modulate aluminum-induced cytokine activation by monocytes *in vitro* and *in vivo* through GPRC6A [31].

Many studies show that Ocn [2,3,32—35] and T [32,36,37] activate GPRC6A in different *in vitro* model systems, including heterologous cells that gain Ocn and T sensing function when transfected with GPRC6A cDNA and cells with endogenous expression of GPRCA that lose Ocn and T activation with knock-down or inhibition of GPRC6A signaling. In addition to its published effects on β-cells, Leydig cells, muscle cells, enteroendocrine L-cells, and prostate cancer cells, Ocn acting through GPRC6A also protects adipocytes and muscle from autophagy and ER-stress and restores insulin sensitivity in high fat diet induced models of insulin resistance [38].

In contrast, several investigators have been unable to demonstrate effects of either Ocn or T to activate GPRC6A in cell culture models

[25,26]. These few negative *in vitro* studies logically cannot disprove the many positive findings; the disparate results likely represent differences in cell models and cDNA constructs used in studies involving transfection of the receptor [32,33]. Nevertheless, the physiological role of Ocn in regulating energy and glucose metabolism remains controversial.

Indeed, questions regarding the functions of Ocn as a ligand for GPRC6A have also been raised. First, Ocn is a dual functioning protein that undergoes post-translational γ-carboxylation of three Glu to Gla residues for anchoring to bone mineral and is de-carboxylated in the acidic environment of bone resorption to generate a ligand for GPRC6A. The carboxylation of Ocn may affect the binding affinity of Ocn for GPRC6A. The domains in Ocn predicted to bind to GPRC6A are the 3-helical tertiary structure of bi-carboxylated Ocn and a 6-amino acid C-terminal region (7). During dissolution of bone mineral, however, undercarboxylated Ocn, γ-carboxylated Ocn, and peptides including a 6-aa C-terminal Ocn peptide lacking a carboxylation site are generated. Initially, either recombinant Ocn or chemically de-carboxlyated native Ocn were shown to activate GPRC6A (34). In other studies, only a small difference in efficacy of uncarboxylated and γ-carboxylated forms of Ocn were observed [19]. In prostate cancer cells, γ-carboxylated Ocn preferentially stimulates prostate cancer cell proliferation [39]. The 6-aa C-terminal peptide can also activate GPRC6A [33]. Thus, the function of Ocn as a ligand can be regulated by post-translational de-carboxylation and cleavage, potentially in a tissue-specific fashion.

Second, Ocn has low overall sequence homology across species, with human Ocn having a 57.1% and 75.5% sequence identity with mouse and rat Ocn, respectively, suggesting that its function may not be conserved across species. Indeed, there are differences in the phenotype of rat and mouse Ocn knockouts, suggesting that functions of Ocn may not be conserved across species. In contrast to the bone and metabolic phenotypes observed in Ocn$^{-/-}$ mice [3,40], Ocn$^{-/-}$ rats have a bone but no metabolic phenotype [41]. The rat Ocn gene structure is closer to humans, and the rat strategy to delete GPRC6A only required targeting a single GPRC6A locus. In contrast, the mouse has a more complex Ocn locus that required deletion of two bone expressed Ocn gene copies and an interspersed adiponectin receptor, paqr6 [3,40], raising the possibility that the metabolic phenotype in Ocn-deficient mice is due to loss of paqr6.

Doubts arising from these inconsistencies are counterbalanced by the pharmacological effects of parenteral administered Ocn to stimulate insulin secretion [6,34], improve glucose tolerance and peripheral insulin sensitivity [42], decrease fat mass [34], and protect against hepatosteatosis in wild-type mice [43,44]. Ocn stimulated GPRC6A functions in β-cells to regulate insulin secretion [33] and in muscle to regulate exercise capacity [20] were lost with organ-specific deletion of GPRC6A. However, in spite of these positive results in animal models, there has been little translational progress in developing Ocn as a valid therapeutic.

Orally administered Ocn can also have effects on insulin release and glucose utilization and protect against the adverse metabolic effects of high fat diets in animals [16,45]. The effect of orally administered Ocn is likely mediated by GPRC6A-dependent effects of Ocn to stimulate the release of the incretin hormone GLP-1 from enteroendocrine L-cells [15]. Unexpectedly, however, male mice administered oral Ocn paradoxically exhibited glucose intolerance and insulin resistance [46]. The basis for sex-dependence in response to oral and parenteral Ocn treatment remains unclear, but T also activates GPRC6A and complex interactions between Ocn, T, and sex hormone binding globulin (SHGB) might occur (*vide infra*).
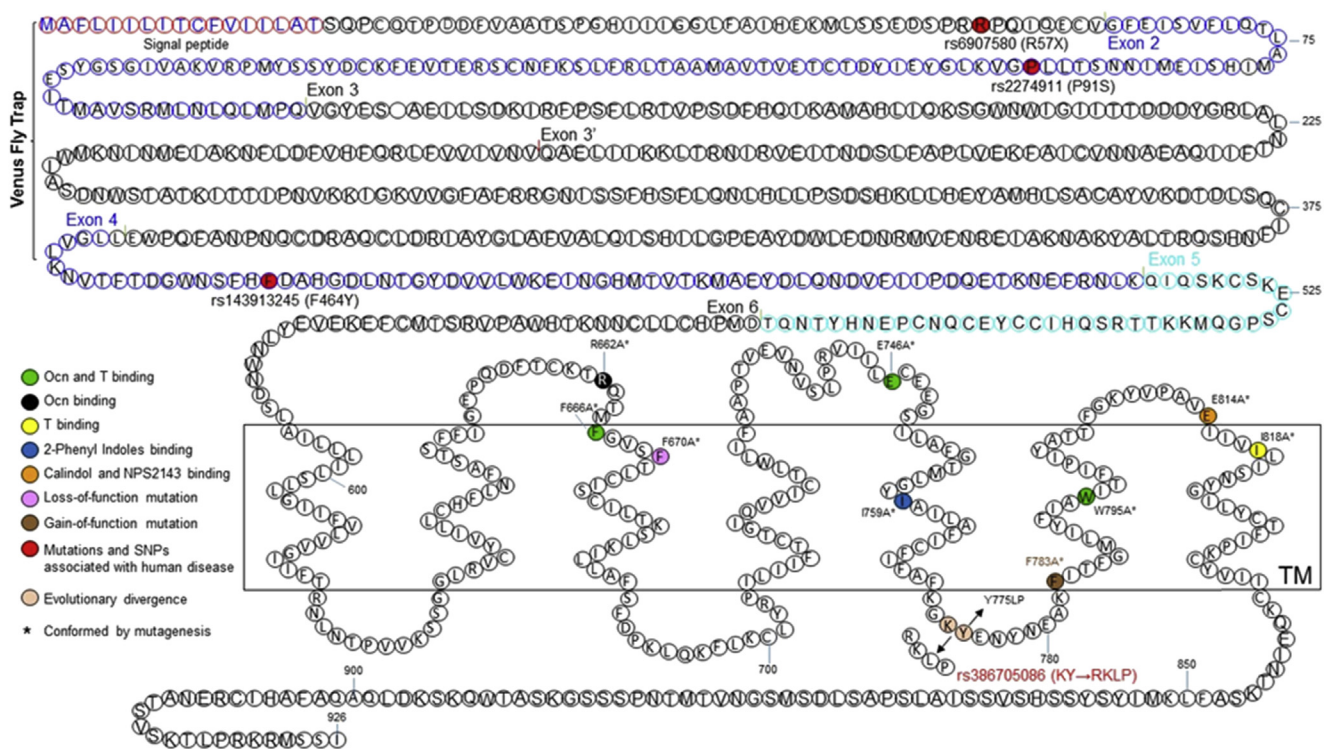
Figure 3: Structure-functional domains of GPRC6A. The schematic shows the amino acid sequence of human GPC6CA that is composed of the venus fly trap (VFT), trans-membrane (TM), and C-terminal domains. The specific amino acid segments encoded by the 6 exons are noted. The position of mutations and SNPs that affect human GPRC6A function are shown in red, the unique Ocn and T binding sites respectively in black, and yellow, and the overlapping Ocn and T sites shown in green. The K..Y is the referent sequence in the 3rd intracellular loop of humans, indicated by, that replaced the ancestral RKLP sequence present in all other species. The RKLP sequence is considered to be the human polymorphism rs386705086 present in 40% of humans of African descent.

More recently, molecular modeling of GPRC6A provides a structural basis for understanding diverse ligand binding to this receptor. GPRC6A consists of a venus fly trap (VFT) motif that is attached to a prototypic 7-transmembrane (7-TM) domain (Figure 2A). There is a general consensus that basic amino acids, such as L-arginine (L-Arg), L-lysine (L-Lys), and L-ornithine (L-Orn) activate GPRC6A through binding to the VFT motif. Binding sites for Ocn are predicted to be in the 7-TM domain and mutations of these sites block the ability of Ocn to activate GPRC6A. The 6-aa carboxyl terminal of Ocn can also activate GPRC6A by binding to the 7-TM site, and amino acid substitutions in this region abrogate activation by Ocn, but not by L-Arg [33]. T is also predicted to bind to epitopes in the 7-TM domain of GPRC6A, and mutagenesis of these sites disrupts T activation of GPRC6A as well. Interestingly, SHBG, which binds T, has homologies to Ocn and is also proposed to bind to GPRC6A and modulate the response to T [37]. Other compounds have been identified that bind to and modulate GPRC6A function. Compounds containing 2-phenyl indoles and calci-mimetics, which activate the related calcium sensing receptor, CaSR, also activate GPRC6A [47]. In addition, mutation of Phe 783 to Ser, which is homologous to an amino acid change that creates a consti-tutively active CaSR [48], also leads to enhanced activation of GPRC6A in response to L-Arg (unpublished observations). Crystal structures of GPRC6A are needed to confirm and expand these computational predictions regarding ligand interactions with GPRC6A.

In addition to the full-length GPRC6A, two other isoforms of GPRC6A have been identified that have alternative splicing of exon 3 encoding amino acids 270—445 and exon 4 encoding 445—516, altering the extracellular domain of this receptor. There is limited information

regarding the effects of these extracellular domain alterations on ligand functions, but the full-length form of GPRC6A is the most highly expressed in metabolic tissues, except the kidney, which expresses equal amounts of isoform 2 [49].

1.3. Diverse tissue functions directly regulated by GPRC6A

The possibility that GPRC6A represents a receptor that can sense multiple, disparate ligands, that potentially interact as positive and negative allosteric modulators, is novel and suggests that this receptor serves as a point of integration of diverse extracellular signals and cellular responses in multiple tissues to create new endocrine net-works. Indeed, GPRC6A is widely expressed, and our current under-standing of its tissue-specific functions largely comes from analysis of the effects of Ocn administration and mouse genetic studies.

Ocn administration acts through GPRC6A to increase pancreatic β-cell number, insulin release, and insulin sensitivity [10,11]. Ocn adminis-tration also stimulates human islets *in vitro* and in xenotransplants to increase β-cell number and insulin release [50]. Ocn signaling through GPRC6A in muscle enables increased adaptation to exercise by stimulating fatty acid and glucose uptake and utilization, as well as regulating muscle mass; and aged mice treated with Ocn increase muscle mass [21].

Mouse genetic studies show that GPRC6A is a recessive gene, since heterozygous mice have no demonstrable phenotype. However, GPRC6A null mice exhibit direct organ-specific defects in glucose and fat metabolism, as well as indirect metabolic effects resulting from GPRC6A regulation of an assortment metabolically active hormones in the mouse. For example, GPRC6A is expressed in pancreatic islet, liver,

skeletal muscle, and fat tissue [7,8,11], in which activation of GPRC6A regulates metabolic processes, including stimulation of insulin secretion and β-cell proliferation [7,8,11], enhancement of peripheral tissue insulin sensitivity, catabolism of glucose and fatty acids in myocytes [8,10,20], and incompletely understood effects to regulate glucose and fatty acid metabolism in liver and adipose tissues. GPRC6A knockout, β-cell and muscle specific GPRC6A knockout mice do not respond to Ocn [11,33], indicating that GPRC6A is required for Ocn effects.

The entire palette of GPRC6A functions is not yet known. Recent studies implicate a role of GPRC6A in muscle functions. Null mice lacking either Ocn or GPRC6A have diminished exercise capability [20,21,33], while a knockout of GPRC6A decreases muscle mass [7]. Conditional deletion of GPRC6A in muscle results in impaired glucose uptake and release of interleukin-6, a myokine that promotes adaptation to exercise [20]. In addition, the ligand for GPRC6A, Ocn, is increased by exercise and may regulate muscle metabolism and muscle mass through GPRC6A [20,21,51]. In contrast, using another GPRC6A null mouse model, others found ablation of GPRC6A increased voluntary wheel running in mice [52].

Although GPRC6A null mice exhibit an increased susceptibility to high fat diet induced obesity, further studies are needed to assess if this is due to direct effects of GPRC6A in adipocytes. However, Ocn has been shown to directly regulate glucose transport as well as TNF-α and adiponectin secretion in primary adipocytes [19]. As noted above, GPRC6A stimulates GLP-1 from intestinal cells [15–17], but the GPRC6A ligand L-Arg moderately stimulated GLP-1 although the stimulation did not reach statistical significance, in GPRC6A null mice, indicating other mechanisms of L-Arg actions [53]. GPRC6A is also expressed in the brain, where existing data suggests that it has central nervous system functions, including regulation of myelin production and seizure thresholds (unpublished data) and LH secretion [7], but there is disagreement if loss of GPRC6A signaling mediates the neurological abnormalities in Ocn$^{-/-}$ mice [1]. In addition, T also stimulates GPRC6A to regulate proliferation in skin keratinocytes [36], and the activation of GPRC6A by T is likely to have other effects attributed to the non-genomic actions of T [28]. There are other tissues, such as the salivary gland, that highly express GPRC6A and for which the function of this receptor is not known. GPRC6A is also expressed in bone and GPRC6A$^{-/-}$ mice exhibit osteopenia and impaired osteoblast mediated bone formation [7], and uncarboxylated Ocn stimulates osteoblast differentiation in murine osteoblasts [11,54,55]; however, another study using a different GPRC6A knockout mouse model failed to show a bone phenotype [56].

GPRC6A may also regulate immune responses. MetS is associated with inflammation, and GPRC6A, which is expressed in monocytes, has been shown to regulate Nlrp3 inflammasome responses in response to aluminum *in vitro* and *in vivo* [57]. GPRC6A is expressed in the myocardium, undercarboxylated Ocn protects against diabetic cardiomyopathy [58], and GPRC6A$^{-/-}$ mice develop a dilated cardiomyopathy (unpublished observations). As noted above, GPRC6A is expressed in the prostate, is increased in human prostate cancer (PCa) cells, and regulates prostate cancer progression in mice [59]. Thus, there are a myriad of physiological functions potentially regulated by GPRC6A and its ligands.

In contrast to the metabolic phenotypes reported in mouse models in which GPRC6A was disrupted [2,3,7,10,11,33,34,61], neither glucose intolerance nor other phenotypes attributed to GPRC6A were reported in two other GPRC6A knockout mouse models [30,62]. Different strategies were used to generate GPRC6A deficient mice, including targeting exons in the extracellular domain [7], encoding the 7TM domain of GPRC6A [29], the entire GPRC6A locus [30]. Deletion of the 7TM, which

leaves intact the extracellular domain of GPRC6A, may act as a dominant negative factor, as reported for a calcium sensing receptor (CASR) knockout model [62], but this does not explain the absence of a metabolic phenotype in the model that completely lacks GPRC6A. Genetic background differences may confound the findings, since the susceptibility to MetS is mouse strain dependent. A more intriguing possibility is that GPRC6A is a recessive gene that confers susceptibility to high fat diets. Indeed, GPRC6A null mice that exhibited no metabolic phenotype on a normal diet exhibit greater increases in basal plasma glucose levels and impairment of oral glucose tolerance and insulin resistance when compared to wild-type mice when placed on a high fat diet [7,63]. Additional studies are needed to determine if GPRC6A represents a susceptibility gene for high fat diet induced metabolic complications in humans, as suggested by these animal studies.

Others have also questioned whether GPRC6A is directly activated by Ocn and T. In contrast to studies showing the ability of Ocn and T to activate GPRC6A, another group found that GPRC6A is activated only by basic amino acid, but not Ocn and T [25]. Ocn, T, SHBG, basic amino acids and cations are simultaneously present in the physiological milieu. At present, it is not clear how these ligands may interact to regulate GPRC6A functions. The VFT and 7-TM binding sites create the potential for both positive interactions and allosteric modulation of GPRC6A activity by ligands binding to these different sites, as well as antagonist effects of ligands competing for the same sites (Figure 3). Several *in vitro* studies found that the human GPRC6A "K..Y" polymorphism, which is present in 84% of humans, is activated by L-Arg, but not by T and Ocn [25,26], suggesting that this polymorphism may have altered the ligand sensing functions of GPRC6A to favor basic amino acids over Ocn and T.

1.4. GPRC6A coupling to multiple signal transduction pathways

Studies have also variably shown coupling of GPRC6A to Gαs-, Gαi- and Gαq-dependent signaling pathways [25,47] (Figure 4). This suggests that GPRC6A may have cell-type specific co-factors that modify its functions. GPRC6A ERK, cAMP, PI3K/Akt/mTOR and AMPK signaling [9,14,32,33].

Some investigators have purported that the K-Y allele is non-functional due to faulty cellular trafficking leading to endosomal localization [26]. Alternatively, the "RKLP" and "K..Y" changes in the 3rd IL might affect β-arrestin binding and the kinetics of membrane trafficking, leading to alterations in the magnitude and/or duration of GPRC6A signaling and/or gain-of-lysosomal signaling due to preferential intracellular localization. A precedent for this comes from the recently discovered endosomal signaling of the PTH GPCR through β-arrestin-dependent mechanism [64]. Thus, the "K..Y" polymorphism in human GPRC6A may have evolved to alter cell trafficking that preferentially leads to activation of mTORC1 intracellular signaling functions. The highly conserved amino acid signaling through mTORC1 occurs in the lysosome, and is mediated by complex cascades involving RAG GTPases, Ragulator, and vacuolar H^{+}-ATPase [65]. It is currently unclear, however, how amino acid sufficiency or limitation is sensed in mammalian cells. This creates an intriguing possibility that humans have evolved changes in GPRC6A as a way to regulate this intracellular amino acid sensing pathway [65] (Figure 4). However, even if the "K..Y" polymorphism proves to be non-functional, the ancestral "RKLP" variant (rs386705086) should have functions and might still explain racial disparities in MetS and PCa.

1.5. Novel endocrine networks created by GPRC6A and its ligands

Although the individual metabolic functions of the multiple hormones stimulated by GPRC6A are well known, the idea that there is a
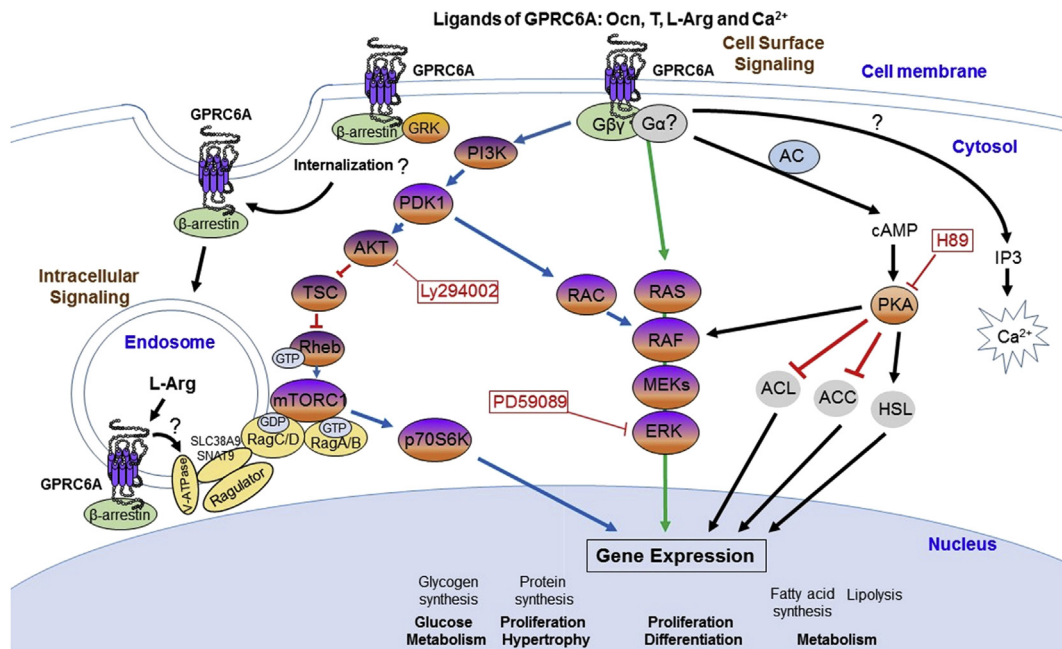
Figure 4: Schematic showing that cell surface GPC6CA acts as a hub that funnels a variety of ligands to give a wide range of intracellular signaling outputs, including activation of PI3K/AKT/mTOR, MAPK, and cAMP-dependent signaling. In addition, there is evidence that GPRC6A with the K..Y polymorphism is predominately located in an intracellular compartment, where it may participate in mTORC1 activation. Further studies are needed to explore whether GPRC6A is coupled to the evolutionarily conserved intracellular pathways that sense amino acids and activate mTORC1 through V-ATPase, Regulator, and RAGA/B/C/D.

coordinated release of insulin [7,8,11], T [2,7], GLP-1 [15—17], adiponectin [18], and IL-6 [20,21] in response to GPRC6A activation is a novel concept.

A surprising number of inter-organ communications and feedback loops are being recognized that are created by GPRC6A-mediated release of hormones [2,3,7,10,11,33,34,61]. For example, Ocn released from bone would directly stimulate insulin, Glp-1, and T release and have direct and indirect effects on muscle, liver, fat, and testes. T activating GPRC6A in Leydig cells would further stimulate its own production and also have peripheral tissue effects to regulate glucose and fat metabolism. T may also act centrally through GPRC6A to suppress LH secretion and disrupt the Ocn-T positive endocrine loop, as evidenced by paradoxical stimulation of LH by T in GPRC6A$^{-/-}$ mice. Insulin and GLP-1 are connected in the fed state, with GLP-1 enhancing insulin release, slowing gastric emptying, and inhibiting glucagon release. Adiponectin administration increases insulin sensitivity, an effect complementary to the increased insulin and GLP-1 induced by Ocn activation of GPRC6A. GPRC6A stimulation in exercising skeletal muscle releases IL-6, which has a feed forward effect to release additional Ocn from bone.

Moreover, since GPRC6A has direct effects on many of the same organs and activates the same signaling pathways that are targeted by these hormones, including ERK, cAMP, PI3K/Akt/mTOR, and AMPK [9,14,32,33], there is potential cross talk between direct and indirect effects of GPRC6A (Figure 4). For example, in the liver, there is overlap between GPRC6A signaling pathways and those regulated by insulin and glucagon. GPRC6A is positioned to impact both insulin signaling through PI3K/Akt and glucagon pathways through activation of cAMP, as well as additional effects through ERK and AMPK activation. Thus, GPRC6A might modulate both pathways, thus serving as a context-dependent enhancer of liver metabolism.

1.6. Clinical relevance of GPRC6A in humans

The functions of Ocn and GPRC6A in humans remain to be established [66]. With regards to Ocn, clinical studies show an inverse correlation between undercarboxylated Ocn levels and obesity, glycemic status, insulin resistance, triglycerides, and leptin and positive correlations with adiponectin [67—69]. Genome wide association studies have linked single nucleotide polymorphisms in the coding and non-coding region of the Ocn gene with body composition in humans [70,71]; a SNP resulting in a R43Q change (rs34702397) in Ocn that is predicted to affect binding to GPRC6A is associated with insulin sensitivity in African Americans [72]. The rs34702397 SNP in Ocn is unevenly distributed in the world population, with 4.65% of people with African, 0.26% of Latino, <0.01% of European, and 0% of Asian descent having the mutant allele (http://exac.broadinstitute.org/variant/6-117150008-G-A).

With regards to the GPRC6A gene, others have questioned if the predicted metabolic functions of GPRC6A are relevant to humans [66], citing the failure to demonstrate membrane location and/or function of the human GPRC6A and high number of SNPs in GPRC6A predicted to inactivate the gene, and the apparent lack of disease associations between these polymorphisms and MetS in humans. Indeed, there are no confirmed Mendelian inherited diseases caused by inactivating mutations of GPRC6A in humans. Although mutations in GPRC6A have been implicated in familial partial lipodystrophy type I (FPLD1), the implicated K774-L-P polymorphism (SNP) and an R57X stop mutation found in FPLD1 is present in 8% of the general population (http://exac.broadinstitute.org/variant/6-117150008-G-A).

The evolutionary changes in GPRC6A provide the strongest evidence for a function of GPRC6A in humans. The sequence RKLP in the 3rd intracellular loop of GPRC6A is present in mice, in which its function has been established, and conserved in all mammalian species except humans, in whom K..Y replaces the RKLP sequence in the majority of

people (60% in African, 85% in Caucasians, and 99% in Asian descent). The RKLP sequence is defined as a variant (rs38670586) in humans and is most commonly found in African-derived populations (40% versus 15% in Caucasians and 1% in Asian descent). More recently, genome-wide association studies (GWAS) show that the P91S (rs2274911) SNP is associated with insulin resistance but not obesity [73—75], the P91S and F464Y SNPs are associated with reduced sperm count and cryptorchidism [73,74], and the GPRC6A genetic locus is associated with C-reactive protein levels [76,77], cardiovascular diseases [78,79], and the risk of PCa in humans [80—82].

2. CONCLUSIONS

If GPRC6A has similar functions in humans as are observed in mice, drugs that activate GPRC6A theoretically would target several abnormalities that cause T2DM, including disordered β-cell function, insulin resistance, and obesity. Indeed, direct effects on β-cells to attenuate loss of β-cell mass, increase insulin and GLP-1 secretion and enhance glucose uptake and insulin sensitivity in peripheral tissues addresses many of the abnormalities underlying MetS. Either an oral or more likely an injectable Ocn, or an orally bioavailable GPRC6A chemical agonist, might be used alone or in combination with existing treatments for T2DM. Potential effects of GPRC6A to ameliorate adverse effects of high-fat diet-induced fatty liver disease and obesity could potentially transform treatments for non-alcoholic fatty liver diseases (NAFLD). On the other hand, activation of GPRC6A may be involved in the pathogenesis of prostate cancer. If so, GPRC6A may be a biomarker for PCa progression and antagonism of GPRC6A could be a novel approach to treat castrate resistant prostate cancer, albeit with the potential side effect of causing MetS.

The preponderance of evidence suggests that GPRC6A is a master regulator of metabolism. GPRC6A is implicated in the pathophysiology of human diseases ranging from metabolic syndrome (MetS) to prostate cancer (PCa) and could provide a molecular understanding of the observed link between MetS and PCa [83]. GPRC6A is a potential therapeutic target in humans, but we remain a long way from developing drugs to activate or antagonize GPRC6A. The therapeutic potential of GPRC6A requires confirmation that GPRC6A's function in mice can be extrapolated to humans, finding ways to selective target this receptor in specific tissues, and establishing the efficacy and safety of drugs that activate or inhibit GPRC6A in pre-clinical trials in animal models of human diseases.

CONFLICT OF INTEREST

None declared.

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