Role of *KCNAB2* expression in modulating hormone secretion in somatotroph pituitary adenoma

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OBJECTIVE Prior profiling of the human pituitary adenoma (PA) DNA methylome showed the potassium channel subunit–encoding gene *KCNAB2* to be highly differentially methylated between nonfunctional PAs (NFPAs) and growth hormone (GH)–secreting PAs, with greater *KCNAB2* methylation detected in secretory PAs. *KCNAB2* encodes an aldo-keto reductase that, among other things, negatively regulates members of the voltage-gated potassium channel (K_v) family. In this study, the authors aimed to determine whether modulation of *Kcnab2* expression would alter GH secretion in the GH3 mammosomatotroph rat cell line. In addition, they examined whether dosing GH3 cells with the antiarrhythmic drug quinidine, a known inhibitor of K_v and voltage-gated sodium channels, would affect hormonal secretion.

METHODS Previously generated RNA-seq data were reanalyzed to compare *KCNAB2* expression levels in human NFPAs and GH-secreting PAs. *Kcnab2* was overexpressed in GH3 cells using plasmid transfection and knocked down using shRNA, with confirmation by quantitative polymerase chain reaction (qPCR). GH concentrations in cell culture supernatants collected 24 hours after cell seeding were measured using enzyme-linked immunosorbent assay (ELISA). Separately, quinidine was administered to GH3 cells at graduated doses. GH and prolactin concentrations in supernatants collected 48 hours after quinidine treatment were measured by fluorometric immunoassay.

RESULTS Modulation of expression at the transcript level in GH3 cells resulted in proportionate changes in the expression of GH mRNA and secretion of GH peptide, as confirmed by qPCR and ELISA. Specifically, partial knockdown of *Kcnab2* was associated with fewer GH RNA transcripts and less GH secretion compared with controls, while augmentation of *Kcnab2* expression was associated with more GH transcripts and secretion than the controls. Administration of quinidine ($\geq 50 \mu\text{M}$) reduced both GH and prolactin secretion in a dose-dependent fashion ($p \leq 0.05$).

CONCLUSIONS GH secretion in a somatotroph cell line is partially dependent on *KCNAB2* gene expression and may be mitigated in vitro by quinidine. These results collectively suggest a potential new target and pharmacological candidate to be considered in the development of clinical therapeutics for acromegaly.

<https://thejns.org/doi/abs/10.3171/2019.12.JNS192435>

KEYWORDS pituitary adenoma; somatotropinoma; neuroendocrine; growth hormone; quinidine; pituitary surgery

PITUITARY adenomas (PAs) are typically benign neoplasms that arise from the anterior pituitary gland, or adenohypophysis. They exhibit an overall prevalence of 16.7% (14.4% in autopsy studies and 22.5% in imaging studies) in the general population. The majority of these tumors are small and nonfunctional (hormonally inactive) tumors or are prolactinomas that can often be successfully treated with dopamine agonist agents. Functional (hormonally active) PAs that secrete ACTH (adrenocorticotrophic hormone) and growth hormone (GH) are responsible for causing Cushing's disease and acromegaly, respectively.⁶ Acromegaly is often an insidious and potentially fatal disease that has myriad systemic effects, including hypertension, diabetes mellitus, cardiovascular disease, pulmonary disease, and the development of cancer, all of which contribute to increased morbidity and mortality. Targeted treatments for refractory acromegaly are still desperately needed when surgery, radiotherapy,

and growth hormone (GH) are responsible for causing Cushing's disease and acromegaly, respectively.⁶ Acromegaly is often an insidious and potentially fatal disease that has myriad systemic effects, including hypertension, diabetes mellitus, cardiovascular disease, pulmonary disease, and the development of cancer, all of which contribute to increased morbidity and mortality. Targeted treatments for refractory acromegaly are still desperately needed when surgery, radiotherapy,

ABBREVIATIONS ELISA = enzyme-linked immunosorbent assay; GH = growth hormone; GHRH = GH-releasing hormone; K_v = voltage-gated potassium channel; NFPA = nonfunctional PA; PA = pituitary adenoma; qPCR = quantitative polymerase chain reaction; RFP = red fluorescent protein.

SUBMITTED September 5, 2019. **ACCEPTED** December 17, 2019.

INCLUDE WHEN CITING Published online February 28, 2020; DOI: 10.3171/2019.12.JNS192435.

and other medical interventions, including somatostatin analogs and pegvisomant, are unsuccessful.

The epigenome, and more particularly the DNA methylome, has recently been implicated in hormonal oversecretion and the invasive growth of PAs. A prior study profiled DNA methylomes of 24 sporadic functional (primarily somatotroph) and nonfunctional PAs (NFPAs), and the authors found that *KCNAB2* was the most differentially methylated gene.¹⁵ More specifically, the body and promoter CpG islands of the *KCNAB2* gene are hypomethylated in somatotroph PAs and hypermethylated in NFPAs.¹⁵ Genes with hypermethylated promoter CpG islands are often transcriptionally inactive.¹⁰ However, the effects on transcription of gene body CpG hypermethylation have yet to be fully elucidated and remain highly contextual.¹⁰

KCNAB2 encodes an aldo-keto reductase^{4,17} that associates as a β auxiliary subunit with the pore-forming α subunits of *Shaker*- and *Shal*-related voltage-gated potassium channels (K_v).¹⁹ Through interaction with the cytoplasmic domains of the α subunits, $K_v\beta 2$ modulates K_v activity,^{21,30} though the operative mechanism has not been conclusively defined.¹⁶ *KCNAB2* is known to play a role in other neuroendocrine conditions.

The relationship between *Kcnab2* and hormone secretion in PAs remains to be determined. Prompted by the findings of our methylome-wide analysis of human PA tissue, we sought to determine the association between *Kcnab2* expression and GH secretion in a somatotroph PA cell line. We found that modulating the expression of *Kcnab2* in the GH- and prolactin-secreting *Rattus norvegicus* PA mammosomatotroph cell line GH3 resulted in corresponding changes in GH expression and secretion. We furthermore found that quinidine, a known inhibitor of K_v and voltage-gated Na^+ channel activity, decreases GH and prolactin secretion from GH3 cells in a dose-dependent manner and may offer an additional avenue for targeted medical treatment in patients with acromegaly.

Methods

PA RNA-Seq Analyses

We used RNA-seq data sets for PA samples, which we generated in a previous study.¹⁵ RNA-seq raw reads (FASTQ files) were mapped to hg38 using STAR 2.7.⁵ Next, we used HTSeq-0.11.2 to transform mapped reads into counts with a human gene annotation file from GENCODE V29 (<https://www.gencodegenes.org/>).⁷ *KCNAB2* gene expression levels were compared between NFPA samples (7 samples that had a high DNA methylation level [$\beta > 0.8$] for the cg18192083 probe, located at the promoter region of *KCNAB2*¹⁵) and GH samples (5 samples that had a low DNA methylation level for the cg18192083 probe) by performing the Wilcoxon rank-sum test after normalizing expression levels by transcript level, calculating transcripts per millions in R (<https://www.r-project.org/>).

Cell Culture

GH3 cells were obtained from the American Type Culture Collection (ATCC) and cultured in Ham's F-12 K Nutrient Mixture, Kaighn's Mod., with L-glutamine (Corning), supplemented with 15% horse serum (HyClone

Equine Sera, GE Healthcare Cell Culture) and 2.5% fetal bovine serum (HyClone). Cells were maintained at 37°C in a humidified 5% CO₂ incubator.

Plasmid Construction, Transfection, and Drug Selection

The multicistronic cassette encoding *R. norvegicus* *Kcnab2*, the P2A sequence, and mCherry was synthesized by the company GENEWIZ. The delivered plasmid containing the cassette was subjected to endonuclease-mediated digestion at the *EcoRV* and *XbaI* sites to excise the cassette. The cassette or a sequence encoding mCherry alone was then cloned into pcDNA3-mRFP (Addgene plasmid #13032, a gift from Doug Golenbock), from which the mRFP insert had been removed. SureSilencing shRNA plasmids encoding shRNAs targeting *R. norvegicus* *Kcnab2* mRNA were obtained from SABiosciences/QIAGEN. The shRNAs that gave successful knockdowns were as follows: clone ID 2, TGAAGTGGTATAGGGAACAT, and clone ID 3, TCCAATGCAGAACAACTAT. The scramble (negative control) shRNA sequence was as follows: ggaatctcattcgatgcatac. Both the pcDNA3 and SureSilencing plasmids contained neomycin-resistance genes. Plasmids were transfected using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. Forty-eight hours later, 500 $\mu\text{g ml}^{-1}$ of G418 sulfate (Gibco), an analog of neomycin, was added to the cell cultures to initiate drug selection. Cell media were replaced every 3–4 days, and G418 was added each time at 500 $\mu\text{g ml}^{-1}$ for 2 weeks, after which it was added at 200 $\mu\text{g ml}^{-1}$ to maintain selective pressure.

Fluorescence-Activated Cell Sorting

Drug-selected GH3 cells expanded from pcDNA3-*Kcnab2*-P2A-mCherry transfectants were sorted based on red fluorescent protein (RFP) expression shown by a BD FACSAria II system running BD FACSDiva 6.1.3 software by the Flow Cytometry Core Facility at the Eli and Edythe Broad CIRM Center for Regenerative Medicine and Stem Cell Research at the University of Southern California. Untransfected GH3 cells were used as RFP-negative controls.

RNA Isolation and Reverse-Transcription and Real-Time Reverse-Transcription qPCR Analysis

Cellular RNA > 200 nt long was isolated using the NucleoSpin RNA Mini Kit (MACHEREY-NAGEL GmbH) according to the manufacturer's instructions. cDNA was generated from eluted RNA using SuperScript IV VILO Master Mix (Invitrogen) according to the manufacturer's instructions. Reverse-transcription products were diluted 1:5 in nuclease-free water prior to use in quantitative polymerase chain reaction (qPCR). Real-time qPCR was performed using the Roche LightCycler 96. PrimeTime qPCR assays and Probe-Based qPCR Master Mix (Integrated DNA Technologies) were used for the reactions. The assays used were as follows: *GH1*, assay ID Rn.PT.58.22100514; *Kcnab2*, assay ID Rn.Pt.58.37057386; and *Mrpl19*, assay ID Rn.PT.58.11051445. The thermocycling parameters for the qPCRs were as follows: 95°C for 1 minute, followed by

35 cycles of 95°C for 15 seconds and 60°C for 1 minute. Relative expression levels were determined by the $2^{-\Delta\Delta CT}$ method with normalization against Mrpl19.

GH Secretion Assay for *Kcnab2* Expression-Modulated Cells

We seeded 10^5 cells per well into a 12-well tissue culture–treated plate (Genesee Scientific) coated with poly-L-lysine. Conditioned media were collected after 24 hours and centrifuged at 10,000g for 5 minutes at 4°C to pellet cells and debris. Supernatants were transferred to low-retention microfuge tubes (Genesee Scientific) and stored at –80°C prior to analysis. Rat GH concentrations were measured using the Rat/Mouse Growth Hormone enzyme-linked immunosorbent assay (ELISA) kit (EMD Millipore) according to the manufacturer's instructions. Each sample was run in duplicate, and absorbance readings were taken at $\lambda = 595$ nm using an Eppendorf PlateReader AF2200 multiwell spectrophotometer. The assay was repeated 3 times.

Cell Viability and Proliferation Determination for Quinidine

We seeded 2×10^4 cells per well into a 96-well standard tissue culture–treated plate (Genesee Scientific) and treated them in triplicate with specified concentrations of quinidine or DMSO (dimethyl sulfoxide) vehicle. The treated cultures were placed in incubation for 45 hours, at which time CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay (MTS; Promega) was administered. Cultures were returned to incubation for an additional 3 hours before absorbance was read at $\lambda = 450$ using an Eppendorf PlateReader AF2200 multiwell spectrophotometer. The assay was repeated 3 times.

Measurement of GH and Prolactin for Quinidine Response Assay

We seeded 10^5 cells per well into a 12-well standard tissue culture–treated plate (Genesee Scientific) coated with poly-L-lysine. Conditioned media of quinidine- or vehicle-treated cultures were collected after 24 hours and centrifuged at 10,000g for 5 minutes at 4°C to pellet cells and debris. Supernatants were transferred to low-retention microfuge tubes (Genesee Scientific) and stored at –80°C prior to analysis. Hormone concentration measurement was performed by the Immune Monitoring Core lab at the University of Southern California Norris Comprehensive Cancer Center using the MILLIPLEX MAP Rat Pituitary Magnetic Bead Panel (EMD Millipore). Samples were run in duplicate from 3 independent quinidine treatments.

Results

We previously identified that the most significant site of differential DNA methylation between NFPA and hormonally active PAs was within the promoter region of the *K_v* regulatory subunit *KCNAB2* (cg18192083).¹⁵ To determine if the DNA methylation levels detected by the cg18192083 probe are related to the gene expression level, we compared *KCNAB2* gene expression levels between 7 NFPA tumors that had a high DNA methylation level at the promoter of the gene and 5 functioning (GH) tumors

FIG. 1. *KCNAB2* gene expression levels in NFPA and GH samples. Graph showing normalized *KCNAB2* gene expression levels (transcripts per million) in 7 NFPA samples that have a high DNA methylation level ($\beta > 0.8$) for the cg18192083 and 5 GH samples that have a low DNA methylation level for the cg18192083 probe. NFA = NFPA; TPM = transcripts per million. $p < 0.15$ (Wilcoxon rank-sum test). Figure is available in color online only.

that had a low DNA methylation level at the promoter of the gene. The average *KCNAB2* gene expression level was lower in NFPA tumors than in GH tumors, but the difference was not statistically significant and possibly underpowered (Fig. 1A) ($p = 0.143$, Wilcoxon rank-sum test).

Prompted by the methylation and expression findings for *KCNAB2*, we decided to investigate the role of this gene in functional PA hormone secretion. The *R. norvegicus* pituitary tumor line GH3 was selected for use in this study due its availability from a reputed repository, robust secretion of GH and prolactin, and extensive citation in the literature. To modulate *Kcnab2* expression in GH3 cells, we transfected them with a plasmid containing a bicistronic *Kcnab2*-P2A-mCherry cassette and a neomycin-resistance gene or plasmids containing one of four shRNA sequences targeting *Kcnab2* and a neomycin-resistance gene (Fig. 2A). Following drug selection, cells were reexpanded and *Kcnab2*-P2A-mCherry cells were subjected to fluorescence-activated cell sorting to enrich for cells with a strong RFP signal (Fig. 2B and C). RFP served as a marker indicating active expression of *Kcnab2* from the *Kcnab2*-P2A-mCherry bicistron. Single cells from the *Kcnab2*-P2A-mCherry and shRNA drug-resistant populations were then isolated and expanded to establish monoclonal lines. Real-time reverse-transcription qPCR was used to detect *Kcnab2* mRNA expression levels. Two *Kcnab2*-P2A-mCherry monoclonal lines with twofold or greater *Kcnab2* expression over controls were selected for further study (Fig. 2D). Of four shRNA designs tested, two were found to give $> 70\%$ *Kcnab2* knockdown (Fig. 2D). Monoclonal populations expressing these shRNAs, designated *Kcnab2* shRNA 2 and *Kcnab2* shRNA 3 to signify the corresponding shRNA design IDs, were utilized for further study.

GH levels in the *Kcnab2*-overexpressing monoclonal lines,

FIG. 2. Modulation of *Kcnab2* expression in GH3 cells. **A:** Linearized representations of pCDNA3-Kcnab2-P2A-mCherry (upper) and pGeneClip with shRNA-encoding sequence (lower) (adapted from vendor-supplied plasmid map), showing crucial regions. bGH poly(A) = bovine GH polyadenylation signal; CMV = human cytomegalovirus immediate early enhancer and promoter; Kcnab2 = coding sequence for *R. norvegicus Kcnab2*; mCherry = sequence encoding a monomeric RFP; Neo = neomycin-resistance gene; P2A = sequence encoding peptide from porcine teschovirus-1 2A; shRNA = sequence encoding SureSilencing shRNA; SV40 = polyomavirus simian virus 40 enhancer and early promoter; SV40 poly(A) = polyadenylation signal; U1 = U1 small nuclear RNA promoter. **B:** Density plots showing gating for red fluorescence-positive and -negative cells. Untransfected GH3 cells were used to set the negative gate (left) prior to flow sorting drug-selected pCDNA3-Kcnab2-P2A-mCherry cells (right). FSC-A = forward scatter; YG = yellow-green laser. **C:** Micrographs of monoclonal cell population pCDNA3-Kcnab2-P2A-mCherry 2. Visible mCherry (right) signifies active expression of the Kcnab2-mCherry bicistron. A similar mCherry signal intensity was observed for Kcnab2-mCherry 3. FLUOR = fluorescence; PHASE = phase contrast. **D:** Real-time reverse-transcription *Kcnab2* expression data for monoclonal GH3 populations stably overexpressing *Kcnab2* (left) or one of two QIAGEN SureSilencing shRNAs (shRNA design 2 or design 3, as indicated) against *Kcnab2* (right). Fold-change values are relative to a pCDNA3-mCherry (left) or scramble shRNA (right) control population. In each case, control expression value = 1. Error bars show the standard deviation from 3 technical replicates. Figure is available in color online only.

Kcnab2-mCherry 2 and Kcnab2-mCherry 3, were found to be elevated compared with pCDNA3-mCherry control (Fig. 3 upper). Conversely, *GHI* expression was reduced in Kcnab2 shRNA 2 and Kcnab2 shRNA 3 compared with scramble shRNA controls (Fig. 3 upper). To assess whether *Kcnab2* and *GHI* expression levels were associated with GH secretion in this model, cells were seeded at equal numbers under standard conditions and aliquots of conditioned cell culture media were collected 24 hours thereafter for ELISA. Elevated *Kcnab2* and *GHI* expression corresponded with higher concentrations of GH in the supernatant compared with controls, whereas subcontrol levels of *Kcnab2* and *GHI* expression corresponded with lower concentrations of GH (Fig. 3 lower). We found no meaningful differences in prolactin concentration associated with *Kcnab2* expression ($n = 1$, data not shown).

We next tested whether the K_v and voltage-gated Na^+ channel modulatory agent quinidine could affect secretion of GH or prolactin from GH3 cells. Used clinically as an

antiarrhythmic and antimalarial drug, quinidine is known to promote inactivation of *Shal*- and *Shaker*-related K_v ,²⁹ which are among the K_v families regulated by *Kcnab2*. To account for antiproliferative and cytotoxic effects of quinidine that could confound assessment of the drug's effect on hormone secretion, we performed a dose-finding viability assay that measured cellular respiration. Statistically significant cellular respiration differences versus control were found at 48 hours for cells treated with $\geq 150 \mu M$ of quinidine (Fig. 4 upper). To minimize this confounding effect, we administered quinidine at $\leq 100 \mu M$ for the hormone secretion assay. We found dose-dependent reductions in both supernatant GH and prolactin concentrations at 24 hours posttreatment (Fig. 4 lower).

Discussion

Here, we have demonstrated that modulating expression of the K_v regulatory subunit *Kcnab2* in an in vitro *R.*

FIG. 3. GH expression and secretion in GH3 populations with modulated *Kcnab2* expression. **Upper:** Real-time reverse-transcription qPCR expression data for *GH1* in monoclonal GH3 populations stably overexpressing *Kcnab2* (left) or one of two indicated QIAGEN SureSilencing shRNA designs against *Kcnab2* (right). Fold-change values are relative to a pCDNA3-mCherry (left) or scramble shRNA (right) control population. In each case, control expression value = 1. Error bars show the standard deviation from 3 technical replicates. **Lower:** GH concentrations in cell-conditioned medium, measured by ELISA 24 hours after cell seeding. Data represent mean $\pm$ SEM of triplicate samples from 3 independent experiments. Statistical significance was calculated using one-way ANOVA with Dunnett's multiple-comparison test in GraphPad Prism version 8. *** $p \leq 0.001$; **** $p \leq 0.0001$.

norvegicus model of functional mammosomatotroph PA results in concordant changes in both the expression of GH mRNA and downstream secretion of GH. This work confirms that *KCNAB2*, a gene that is widely differentially methylated between NFPA and functional PAs, plays a role in GH secretion. We have also shown that the K_v modulatory drug quinidine negatively regulates the emission of both GH and prolactin in vitro from GH3 cells in a dose-dependent fashion.

FIG. 4. Effect of quinidine on GH3 cell viability and hormone secretion. **Upper:** MTS assay readings 48 hours after treatment of GH3 cells with indicated concentrations of quinidine. Lower absorbance (Abs) values relative to control values indicate less cellular respiration, due to loss of viability or suppressed cell proliferation. Vehicle control = 0. ANOVA F value = 13.68, degrees of freedom = 23. **Lower:** Relative GH and prolactin concentrations in conditioned medium of cells treated with indicated concentrations of quinidine, measured by ELISA 24 hours after drug administration. Vehicle control = 0. **Left:** ANOVA F value = 13.67, degrees of freedom = 11. **Right:** F value = 22.74, degrees of freedom = 11. For all graphs, data represent mean $\pm$ SEM of triplicate samples from 3 independent experiments. Statistical significance was calculated using one-way ANOVA with Dunnett's multiple-comparison test in GraphPad Prism version 8. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

The mechanism by which *Kcnab2* influences GH secretion remains to be fully elucidated. In normal somatotropes, GH secretion is stimulated by the binding of GH-releasing hormone (GHRH) or ghrelin to cognate receptors situated in the cytoplasmic membrane.^{8,13} GH3 cells lack GHRH receptors but constitutively secrete GH,¹⁴ recapitulating the autonomous production of GH by somatotropomas that underlies most cases of human acromegaly.¹⁸ GH release in somatotropes is preceded or accompanied by cell membrane depolarization,⁸ and GH3 cells undergo rapid cycles of dysregulated depolarization and repolarization.¹¹ *Shaker*- and *Shal*-related K_v play a role in repolarization,²⁸ suggesting that the *Kcnab2*-associated effect on GH output described herein is likely attributable to *Kcnab2*-mediated modulation of repolar-

ization. The mechanism by which *Kcnab2* expression affects hormone production and/or secretion is beyond the scope of the current work and will be focused on in future studies that will necessitate patch clamping and potassium current recording.

The physiologic relationship between $K_v\beta 2$ and $K_v\alpha$ begins prior to pore assembly, at the time of translation, when $K_v\beta 2$ promotes cotranslational glycosylation of developing *Shaker*-related $K_v1.2$ polypeptides.²⁷ Immediately subsequent to this event, $K_v\beta 2$ performs further chaperone-like functions that promote expression of $K_v1.2$ in the cytoplasmic membrane.^{12,27} $K_v\beta 2$ also plays a role in regulating cell surface expression of K_v1 through a mechanism in which Cdk-mediated phosphorylation of $K_v\beta 2$ in microtubule-associated $K_v\beta 2$ - K_v1 complexes triggers dissociation of $K_v\beta 2$ - K_v1 from the microtubule adapter protein EB1, allowing $K_v\beta 2$ - K_v1 to translocate to the plasma membrane and multimerize into functional channels.³¹ $K_v\beta 2$ associates with entities other than $K_v\alpha$, including TRPV1, an ion channel in dorsal root ganglia that plays a role in thermal and inflammatory pain perception,³ and ProSAP2, a postsynaptic density anchoring molecule in primary hippocampal neurons.²⁴ The range of interacting partners also extends beyond proteins, as $K_v\beta 2$ has been shown to bind the long noncoding RNA NEAT1 in the nuclei of neuroblastoma cells.² $K_v\beta 2$ is abundant in mammalian brain^{22,25} and heart,²³ contributing to the regulation of excitability in both organs through interaction with K_v . Ablation of *Kcnab2* in mice results in diminished associative learning and memory, as well as amygdala hyperexcitability.²⁰ A gain-of-function mutation in *KCNAB2* is associated with Brugada syndrome, a cardiac arrhythmia disorder.²³ Phosphorylation and acetylation of $K_v\beta 2$ and $K_v1.5$ are upregulated, and binding of $K_v\beta 2$ to $K_v1.5$ is enhanced in pancreatic β cells stimulated with gastrointestinal incretin hormones.¹²

Given that *Kcnab2* interacts with entities other than K_v , the observed GH effects might be a result of yet-unreported interactions with the pathway that regulates GH expression. Stimulation of the G protein-coupled GHRH receptor by GHRH leads to an increase in intracellular cAMP, activation of protein kinase A, and elevated expression of Pou1f1, which associates with phosphorylated cAMP response element binding protein, or CBP, to cooperatively induce expression of *GH*.^{1,26} *Kcnab2* might interact with Pou1f1 or CBP or entities upstream of them in the signaling pathway to influence GH expression and biosynthesis. Additional work is needed to articulate the mechanism by which *Kcnab2* modulates GH secretion. Moreover, studies employing somatotropes and somatotropinoma-derived cells of other provenances must be conducted to ascertain whether the *Kcnab2*-associated effect on GH secretion is idiosyncratic to GH3 cells.

Quinidine is a K_v and Na^+ channel inhibitor and has been used for decades in the clinical treatment of arrhythmia and malaria. Our finding that quinidine suppresses GH and prolactin secretion in GH3 cells in a dose-dependent manner indicates the potential of this drug as a treatment for acromegaly and, potentially, hyperprolactinemia. Additional *in vitro* and *in vivo* work will be needed that confirms the ability of quinidine to mitigate GH and prolactin

hypersecretion in more than just GH3 cells. This work, however, remains limited by a paucity of available human PA cell lines with which to sufficiently test this intervention. Nonetheless, the quinidine-associated effects we observed also underscore the central role of ion channel activity in determining the secretory behavior of endocrine cells. Indeed, in our study of DNA methylation in PAs, we found the ion channel activity signaling pathway to be the most differentially methylated gene set of all sets analyzed between functional (somatotroph) and nonfunctional PAs.¹⁵ Quinidine modulates action potentials in heart cells by slowing the rate of repolarization.⁹ We therefore reason that the reduced hormonal emission from quinidine-treated GH3 cells results from a diminution in the frequency of their spontaneous action potentials.¹¹ Prior administration of quinidine to human adult and pediatric populations did not show a major effect on growth or GH levels, but this may be limited by short-term use, therapeutic levels, or a lack of testing of this axis, in which a deficiency in adults may have nonspecific and nonlethal effects.

Limitations and Future Directions

The current study is limited by its work exclusively in a rat cell line, without associated human somatotroph cell line validation. It is inherently limited by the lack of a sustainable, expandable human somatotroph cell line in which to perform this work. Nevertheless, we believe that identification of this widely differentially methylated gene in human PAs and the dose-dependent relationship observed with *KCNAB2* modulation *in vitro* hold significant potential for future treatment using selective methylation inhibitors or targeted K_v channel inhibitors that have already been shown to be safe in humans (e.g., quinidine). Future work will help to elucidate the precise mechanisms underlying *KCNAB2*-mediated hormonal secretion in human PAs. Clinical testing with dose escalation may be warranted in the future to determine whether the *in vitro* findings presented here translate to human populations with acromegaly.

Conclusions

We have shown that the expression level of *Kcnab2* correlates with intracellular expression of GH mRNA and downstream emission of GH peptide in a rat cell model of PA with up- or downmodulated *Kcnab2* expression. We also demonstrated that the antiarrhythmic and antimalarial drug quinidine dose dependently reduces pathological hormone secretion in the same model of PA. This work elucidates a heretofore unreported role for an ion channel regulatory subunit in regulating hormone secretion in endocrine cells. Our results for quinidine suggest this small molecule as a potential means of controlling acromegaly.

Acknowledgments

We thank Dr. Qi-Long Ying of the Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at USC and Dr. Chang Tong of OCgene Therapeutics Corporation for helpful conversations.

Support for this work comes from NIH grant R01 CA230328 01 (to G.Z.).

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Ashton, Carmichael, Zada. Acquisition of data: Ashton. Analysis and interpretation of data: Ashton, Rhie. Drafting the article: Ashton, Zada. Critically revising the article: Carmichael, Zada. Reviewed submitted version of manuscript: Rhie, Carmichael, Zada. Approved the final version of the manuscript on behalf of all authors: Ashton. Statistical analysis: Ashton, Rhie. Administrative/technical/material support: Ashton. Study supervision: Zada.

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