Stable GPR101 over-Expressing Cell Lines As an Invaluable Tool for Functional Studies, Ligand Screening, and the Identification of Deregulated Genes/Pathways in Patients with X-Linked Acrogigantism

Number: SUN 269
Category: Gene Regulation & Development

Authors

Giampaolo Trivellin PhD (/tristar_endo17/speaker/9f6300891abea35983bbd25dffa74079)

Body

Giampaolo Trivellin[*1], Marija M Janjic[1], Darwin Omar Larco[2], Melanija Tomic[1], Adrian F Daly[3], Leonor Palmeira[3], Fabio R. Faucz[1], Albert Beckers[3], T John Wu[2], Davide Calebiro[4], Stanko S Stojilkovic[1] and Constantine A Stratakis[1]
[1]Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD, [2]Uniformed Services University, Bethesda, MD, [3]CHU de Liège-University of Liège, Liège, Belgium, [4]University of Würzburg, Würzburg, Germany

Background: GPR101 is an orphan G protein-coupled receptor (GPCR) that is duplicated in patients with X-linked acrogigantism (X-LAG) and over-expressed in their GH- and PRL-secreting tumors. GPR101 is a constitutively active GPCR that strongly activates the cAMP pathway. To elucidate the mechanisms through which GPR101 causes GH over-secretion we generated HEK293 and GH/PRL-secreting (GH3) cells with stable GPR101 expression.
Methods: Both cell lines were created via direct integration of a human *GPR101*-coding sequence into their genome. In HEK293 cells this was achieved by transient transfection of a *GPR101*-expressing plasmid, while GH3 were transduced with GPR101 lentiviral particles. Cells were selected with appropriate antibiotics and the surviving clones expanded. GPR101 expression was quantified by RT-qPCR and immunofluorescence/western blotting. Cell proliferation (MTT assay), cAMP levels (^{125}I-labeled cAMP tracer), and calcium signaling (FURA 2 AM) were determined. RNA was extracted from both cell lines and subjected to RNA-seq. Differential gene expression between control and GPR101-expressing cells and pathway analysis was carried out with the Stirplate and MetaCore softwares, respectively. De-regulated genes were validated by RT-qPCR.

Results: High GPR101 expression was achieved in both cell lines and confirmed at the mRNA and protein level. *GPR101*-expressing cells proliferated at different rates from the respective controls: GPR101-HEK293 cells were slow-dividing, while GPR101-GH3 divided faster. cAMP production was enhanced in GPR101-GH3 and accompained by increased excitability of cells. Differential expression analysis in HEK293 cells revealed several up-regulated and few down-regulated genes. Among the genes with high expression, several were linked to the cAMP pathway: *CGA*, *PCK1*, *LINC00473* and *PDE3A*. Enrichment analysis ranked cytoskeleton remodeling and cell cycle regulation (inhibition of G1/S transition) as the most relevant pathways. In GH3 cells most of the genes

with a significantly different expression encoded for membrane-localized proteins, among which were ion channels (*Trpm8*, *Kcnj1*), GPCRs (*Trhr*), and calcium sensors (*Syt4*, *Anxa1*). Biological processes associated with these genes are: vesicle transport and fusion, cytoskeleton organization, and energy homeostasis.

Conclusions: These results show that the intrinsic activity of GPR101 strongly stimulates cAMP production and this in turn facilitates voltage-gated calcium influx. Changes in cAMP/calcium signaling are accompanied with faster/slower cell division depending on the cell type. Accordingly, several genes associated with these and related pathways are differentially expressed. The establishment of these cell lines will be of paramount importance to validate putative GPR101 ligands and to conduct functional studies.

Nothing to Disclose: GT, MMJ, DOL, MT, AFD, LP, FRF, AB, TJW, DC, SSS, CAS

Please take note of the Endocrine Society's News Embargo Policy at: https://www.endocrine.org/news-room/endo-annual-meeting (https://www.endocrine.org/news-room/endo-annual-meeting)

Sessions

SUN 244-273 Cellular Signaling in the Endocrine System
Sunday, Apr 02 1:00 PM
OCCC - West Hall B (EXPO Hall)
(/tristar_endo17/event/9f6300891abea35983bbd25dff577ef9)