Characterization of GPR101 transcripts structure, expression, and signaling

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Background: Patients with growth hormone (GH)-secreting pituitary tumors leading to early childhood-onset gigantism were recently found to harbor germline Xq26.3 duplications including *GPR101*. GPR101, an orphan GPCR for which little is known, is highly expressed in the tumors of the patients.
Methods: We characterized GPR101 transcripts *in vitro* in human tissues by integrating 5'-RACE and RNAseq, and we predicted the putative promoter region *in silico*. GPR101 expression was investigated at the mRNA and protein level in post-mortem human, rat, and zebrafish tissues, by qPCR, whole-mount *in situ* hybridization, and immunostaining. GPR101 signaling was studied in HEK293 and GH-secreting (GH3) cells by using luciferase reporter assays and fluorescence resonance energy transfer (FRET) imaging.
Results: Two GPR101 isoforms have been identified, characterized by different 5' UTRs and a common 6.2 kb-long 3'UTR. A CpG-enriched promoter region was predicted within 1 kb upstream of the putative transcription start site. GPR101 is expressed at low or no levels in almost all adult human tissues except for specific regions of the brain. Additionally, high GPR101 expression was seen in human fetal pituitary. GPR101 was also expressed in several brain areas during zebrafish and rat development. While GPR101 over-expression strongly activates the cAMP pathway in basal conditions, only a very modest increase in Gi- and no activation of Gq-mediated pathway was seen.
Conclusions: This study shows that different GPR101 transcripts exist and that the brain is the major site of GPR101 expression across different species, suggesting an important role in brain/hypothalamic functions. GPR101 has high basal constitutive activity by acting mainly through the cAMP pathway, for which mitogenic effects in GH-secreting cells are well established.