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Research Report

Characterization of *Gpr101* expression and G-protein coupling selectivity

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ABSTRACT

This report describes the identification and characterization of the murine orphan GPCR, *Gpr101*. Both human and murine genes were localized to chromosome X. Similar to its human ortholog, murine Gpr101 mRNA was detected predominantly in the brain within discrete nuclei. A knowledge-restricted hidden Markov model-based algorithm, capable of accurately predicting G-protein coupling selectivity, indicated that both human and murine GPR101 were likely coupled to Gs. This prediction was supported by the elevation of cyclic AMP levels and the activation of a cyclic AMP response element-luciferase reporter gene in HEK293 cells over-expressing human GPR101. Consistent with this, over-expression of human GPR101 in a yeast-based system yielded an elevated, agonist-independent reporter gene response in the presence of a yeast chimeric Gαs protein. These results indicate that GPR101 participates in a potentially wide range of activities in the CNS via modulation of cAMP levels.

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1. Introduction

The recent identification of numerous G-protein-coupled receptors (GPCRs) in the mammalian genome has created a large catalog of uncharacterized receptors (Vassilatis et al., 2003). This presents the significant challenge of identifying cognate ligands for these numerous orphan GPCRs (oGPCRs). Investigators have used a diversity of approaches to identify endogenous ligands for oGPCRs including bioinformatic methods applied to oGPCR sequences, searching sequence databases for likely peptide ligands, testing panels of biologically active compounds and screening fractionated tissue extracts in a process referred to as reverse pharmacology (Civelli et al., 2001; Lee et al., 2003; Wise et al., 2004). In reverse pharmacology approaches, oGPCRs are first expressed in cell lines that permit efficient functional expression followed by detection of G-protein- and agonist-dependent activation by various methods. These methods take advantage of well-characterized signal transduction pathways with robust downstream readouts, such as ^{35}S-GTPγS binding, second messenger production (e.g. cAMP, inositol phosphates, intracellular Ca^{2+}) and second messenger-dependent, G-protein-coupled reporter genes. Using these systems, oGPCR ligands have been identified by screening known compound panels prioritized based on the similarity of oGPCRs to GPCRs with known ligands (Briscoe et

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al., 2003; Brown et al., 2003; Inbe et al., 2004; Itoh et al., 2003; Kotarsky et al., 2003; Liu et al., 2001; Morse et al., 2001; Nakamura et al., 2000; Nguyen et al., 2001; Nilsson et al., 2003; Oda et al., 2000; Zhu et al., 2001). Additionally, such assays have been employed to screen biological extracts of tissues selected based on the expression pattern of the oGPCR (Hinuma et al., 1998; Kojima et al., 1999; Liu et al., 2003; Meunier et al., 1995; Reinscheid et al., 1995; Saito et al., 1999; Sakurai et al., 1998; Shimomura et al., 1999, 2002; Zhang et al., 2001).

The choice of a reverse pharmacology signal transduction assay can be tailored to the oGPCR if specific G-protein coupling selectivity can be determined. Therefore, deciphering coupling selectivity is an important step in understanding the biology of an oGPCR and in the development of cell-based assays for discovery of natural or surrogate ligands. Because the coupling specificity of many oGPCRs has yet to be experimentally determined, various computational strategies have been developed (Cao et al., 2003; Moller et al., 2001; Sreekumar et al., 2004). These approaches have proven to be quite accurate in defining the G-protein coupling selectivity of GPCRs with known ligands.

A recent report described the identification of the human oGPCR, GPR101 (Lee et al., 2001). Alignment of this sequence with all known GPCRs predicts that it is most similar to another orphan receptor, *GPR161* (also known as *RE2*), and falls into a class of orphan GPCRs whose closest relatives with known ligands are the melatonin receptors and the opsins (Vassilatis et al., 2003). However, the similarity between these receptors is low, making prediction of an endogenous ligand based on sequence similarity alone unlikely. *GPR101* is also weakly related to the histamine H2

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                 .         .         .         .         .
Human    1 MTSTCTNSTRESNSSHTCMPLSKMPISLAHGIIRSTVLVIFLAASFVGNI 50
           |  .|||||.|.| |   |:||||||||.||||||| ||.: |  .|.||:
Mouse    1 MPPSCTNSTQENNGSRVCLPLSKMPISVAHGIIRSVVLLVILGVAFLGNV 50
                 .         .         .         .         .
        51 VLALVLQRKPQLLQVTNRFIFNLLVTDLLQISLVAPWVVATSVPLFWPLN 100
           ||  || ||| |||||||||||||||||:.|||||||.|.:| |||||
        51 VLGYVLHRKPNLLQVTNRFIFNLLVTDLLQVALVAPWVVSTAIPFFWPLN 100
                 .         .         .         .         .
       101 SHFCTALVSLTHLFAFASVNTIVVVSVDRYLSIIHPLSYPSKMTQRRGYL 150
            |||||||||||||||||||||||||||||.|||||||||||| || |:
       101 IHFCTALVSLTHLFAFASVNTIVVVSVDRYLTIIHPLSYPSKMTNRRSYI 150
                 .         .         .         .         .
       151 LLYGTWIVAILQSTPPLYGWGQAAFDERNALCSMIWGASPSYTILSVVSF 200
           ||||||| | |||||||||||| | ||:||| |||||||||.||:.|||||
       151 LLYGTWIAAFLQSTPPLYGWGHATFDDRNAFCSMIWGASPAYTVVSVVSF 200
                 .         .         .         .         .
       201 IVIPLIVMIACYSVVFCAARRQHALLYNVKRHSLEVRVKDCVENEDEEGA 250
           :|||| |||||||||| ||||| ||||  | | |||||.| | .|.||||
       201 LVIPLGVMIACYSVVFGAARRQQALLYKAKSHRLEVRVEDSVVHENEEGA 250
                 .         .         .         .         .
       251 EKKEEFQDESEFRRQHEGEVKAKEGRMEAKDGSLKAKEGSTGTSESSVE. 299
           .|::||||..||. |  |     .|    .:  : |.  |  | . |.:
       251 KKRDEFQDKNEFQGQDGGGQAEAKGSSSMEESPMVAEGSSQKTGKGSLDF 300
                 .         .         .         .         .
       300 ARGSEEVRESSTVASDGSMEGKEGSTKVEENSMKADKGRTEVNQCSIDLG 349
           . |  | ::|  | |.||||| |  |. : .| ||| || : |||.||.|
       301 SAGIMEGKDSDEV.SNGSMEGLEVITEFQASSAKADTGRIDANQCNIDVG 349
                 .         .         .         .         .
       350 EDDMEFGEDDINFSEDDVEAVNIPESLPPSRRNSNSNPPLPRCYQCKAAK 399
           |||.||| |:|.|. |||||. |||| ||||||| |.|||| ||:||||:
       350 EDDVEFGMDEIHFN.DDVEAMRIPESSPPSRRNSTSDPPLPPCYECKAAR 398
                 .         .         .         .         .
       400 VIFIIIFSYVLSLGPYCFLAVLAVWVDVETQVPQWVITIIIWLFFLQCCI 449
           |||:|| .|||||||||||||||||::|.|||||||||||||||||||||
       399 VIFVIISTYVLSLGPYCFLAVLAVWVDIDTRVPQWVITIIIWLFFLQCCI 448
                 .         .         .         .         .
       450 HPYVYGYMHKTIKKEIQDMLKKFFCKEKPPKEDSHPDL....PGTEGGTE 495
           ||||||||||.||||||:.|||  ||. || |||||||     ||||| |
       449 HPYVYGYMHKSIKKEIQEVLKKLICKKSPPVEDSHPDLHETEAGTEGGIE 498
                 .
       496 GKIVPSYDSATFP 508
           || |||:|||| |
       499 GKAVPSHDSATSP 511
```

Fig. 1 – BestFit alignment of human (NM_054021) and murine *GPR101*. Vertical lines indicate identical amino acids. Double dots indicate conserved substitutions, single dots semi-conserved. The two proteins are 70.75% identical as determined by BestFit.

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