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Research paper

Mutational mapping of the transmembrane binding site of the G-protein coupled receptor TGR5 and binding mode prediction of TGR5 agonists

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ABSTRACT

TGR5 (Gpbar-1, M-Bar) is a class A G-protein coupled bile acid-sensing receptor predominately expressed in brain, liver and gastrointestinal tract, and a promising drug target for the treatment of metabolic disorders. Due to the lack of a crystal structure of TGR5, the development of TGR5 agonists has been guided by ligand-based approaches so far. Three binding mode models of bile acid derivatives have been presented recently. However, they differ from one another in terms of overall orientation or with respect to the location and interactions of the cholane scaffold, or cannot explain all results from mutagenesis experiments. Here, we present an extended binding mode model based on an iterative and integrated computational and biological approach. An alignment of 68 TGR5 agonists based on this binding mode leads to a significant and good structure-based 3D QSAR model, which constitutes the most comprehensive structure-based 3D-QSAR study of TGR5 agonists undertaken so far and suggests that the binding mode model is a close representation of the "true" binding mode. The binding mode model is further substantiated in that effects predicted for eight mutations in the binding site agree with experimental analyses on the impact of these TGR5 variants on receptor activity. In the binding mode, the hydrophobic cholane scaffold of taurolithocholate orients towards the interior of the orthosteric binding site such that rings A and B are in contact with TM5 and TM6, the taurine side chain orients towards the extracellular opening of the binding site and forms a salt bridge with $R79^{EL1}$, and the 3-hydroxyl group forms hydrogen bonds with $E169^{5.44}$ and $Y240^{6.51}$. The binding mode thus differs in important aspects from the ones recently presented. These results are highly relevant for the development of novel, more potent agonists of TGR5 and should be a valuable starting point for the development of TGR5 antagonists, which could show antiproliferative effects in tumor cells.

1. Introduction

TGR5 (Gpbar-1, M-Bar) is a class A G-protein coupled receptor (GPCR) signaling via a stimulatory G protein and is activated by both unconjugated and conjugated bile acids and various steroid hormones including neurosteroids [1–3]. TGR5 is widely expressed in humans and rodents; organs with high amounts of TGR5 mRNA expression include the brain, the liver, and the gastrointestinal tract [1,2,4,5]. In liver, TGR5 modulates hepatic microcirculation, exerts anti-inflammatory, anti-apoptotic and choleretic effects, and promotes gallbladder filling [6–9]; in the intestine, TGR5 activation in L-cells has been linked to increased secretion of the insulin response-modulating glucagon-like peptide-1. Administration of TGR5 agonists reduced liver inflammation and steatosis and improved glucose tolerance in animal models [10]; furthermore, a reduction of atherosclerotic plaque formation was observed [11]. This makes TGR5 a promising drug target for the treatment of metabolic disorders, such as non-alcoholic steatohepatitis, type II

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diabetes, obesity, and atherosclerosis [11–13]. Accordingly, much effort has been devoted to the development of potent and selective agonists of TGR5 [14–18]. Due to the lack of a crystal structure of TGR5, the development has been guided by ligand-based approaches [3,14–24] so far.

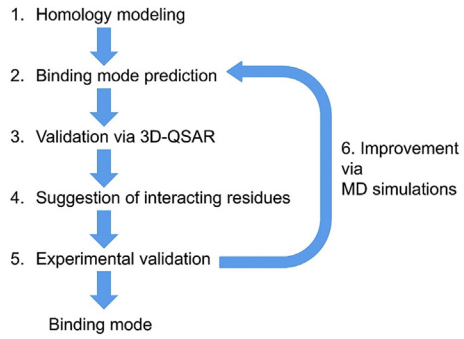
Only very recently, an integrated computational, biological, and chemical approach was presented by Macchiarulo et al. with the aim to probe the transmembrane binding site of TGR5 by mutational analysis and to predict the binding mode of agonistic bile acids and derivatives [19]. The computational part was based on a homology model of human TGR5 derived from a template structure of rhodopsin in the inactive state, with refinement of some of the binding site residues by energy minimization [19]. This resulted in the identification of "binding mode 3" [19] compliant to most of the mutagenesis data. In "binding mode 3" [19], bile acids are oriented in a head-to-tail fashion with respect to transmembrane helix (TM) 3, with the 3-hydroxyl group being involved in hydrogen-bonding interactions with N93 (position 3.33, Ballesteros–Weinstein nomenclature [25] according to the GPCR database [26], hereafter abbreviated as $N93^{3.33}$) and $Y89^{3.29}$. However, this binding mode does not explain why $E169^{5.44}$ (note that in the work of Macchiarulo et al. this residue is referred to as $E169^{5.53}$) [19], implicated to be a key residue from the degree of conservation in a TGR5 sequence alignment [19], led to a reduced TGR5 activation upon Glu169Ala mutation. Agonists in "binding mode 3" are more than 12 Å away from $E169^{5.44}$ so that it is difficult to envisage how an agonist would sense this mutation. Comparison of an active state of an agonist-bound β_2 -adrenergic receptor (β_2AR) with an inactive, antagonist-bound β_2AR state may provide an explanation for this: It revealed an inward bulge of TM5 centered around position 5.46 as the greatest structural difference in the binding pocket of the active state with a position shift of the C_α atom by 2.1 Å [27]. In addition, smaller inward movements of TM6 and TM7 were observed [27]. Such movements may influence the success of docking to a rigid TGR5 model as used by Macchiarulo et al. for agonist placement [28–30]. Furthermore, a homology model of TGR5 generated by us (see below) showed that $N93^{3.33}$ favors a conformation pointing away from the binding site such that hydrogen bond formation with the 3-hydroxyl group of bile acid derivatives, as postulated by Macchiarulo et al. [19], appears less likely. Finally, "binding mode 3" does not interact with TM6 [19]. However, interactions between agonists and TM6 are considered essential for GPCR activation [31–35]. Another two binding mode models of bile acid derivatives in a structural model of TGR5 have been presented by D'Amore et al. [36] and Yu et al. [37], both being based on combinations of molecular docking and molecular dynamics (MD) simulations applying *a priori* restraints to guide the ligand placement. Both binding modes differ from the one proposed by Macchiarulo et al., either with respect to the overall orientation of the bile acid derivative [37] or with respect to the location and interactions of the cholane scaffold [36]. In contrast to the study by Macchiarulo et al. [19] and the present study, no mutagenesis studies were performed by D'Amore et al. [36] and Yu et al. [37] to confirm the proposed binding modes.

These circumstances prompted us to predict a binding mode for natural and synthetic bile acids and neurosteroids starting from a structural model of TGR5 generated from multiple GPCR template structures and to perform mutational mapping of the transmembrane binding site of TGR5 to validate these predictions. In this process, we relaxed the TGR5 model in the presence of an agonist by all-atom MD simulations in an explicit membrane environment. Finally, we derived a protein-based 3D-QSAR model for 68 TGR5 agonists, including both bile acids and neurosteroids, with good predictive power based on the binding mode. With respect to the studies of Macchiarulo et al. [19], D'Amore et al. [36], and Yu et al. [37] our binding mode model differs in one or more of the following five aspects: I) The ligands in our binding mode are oriented parallel to the membrane, rather than perpendicular to it as in the binding mode of Yu et al. [37]; II) the cholane scaffold of bile acids binds in the vicinity of TM5 and TM6 and is rotated by 180° around the long axis compared to Macchiarulo's "binding mode 3" [19]; III) the 3-hydroxyl group of bile acids interacts with the conserved $E169^{5.44}$ [19], but neither with $N93^{3.33}$ nor with $W237^{6.48}$, which is in contrast to "binding mode 3" and the binding mode by Yu et al. [37]; IV) through this, our binding mode provides an explanation for the observed selectivity towards epimers for bile acids with a 7-hydroxyl group; V) the side chains of bile acids orient towards EL1 and, hence, are distant from $S270^{7.43}$, in contrast to "binding mode 3" [19] and the binding mode of D'Amore et al. [36].

2. Results

We pursued an iterative and integrated computational and biological approach to elucidate the binding mode of agonistic TGR5 bile acids and neurosteroids (Scheme 1); similar approaches have been successfully applied to other GPCRs [38–41]. After generating multiple structural models of TGR5 by homology modeling (step 1), initial binding modes of these models were predicted by molecular docking (step 2). The binding modes were evaluated using the predictive power of structure-based 3D-QSAR analyses as a quality criterion (step 3). Based on the best binding mode, potentially interacting residues were predicted (step 4). For experimental validation, variants of TGR5 with single-point mutations of these residues were generated, and the influence of the mutations was investigated with respect to plasma membrane localization and function using immunofluorescence staining, flow cytometry, and a cAMP responsive luciferase assay (step 5). To further improve the binding mode, the TGR5/agonist complex was relaxed by MD simulations (step 6), whereupon steps 2 to 5 were repeated to reach the final binding mode. These steps will be described in detail in the following.

Step 1 – Homology modeling of TGR5. In order to generate a structural model of TGR5, we applied a multi-template homology modeling approach. All antagonist-bound class A GPCR crystal structures with a resolution <3 Å available at the beginning of this study and identified by a PSI-BLAST [42] search with the TGR5 sequence as a query served as templates. If more than one structure matched these criteria for a GPCR, the structure with the best resolution was chosen. This resulted in seven templates, the turkey β_1-adrenergic receptor (PDB code 2VT4), the human β_2-adrenergic receptor (3D4S), the human adenosine-A_{2A} receptor (3EML), the human CXCR4 receptor (3ODU), the human dopamine-D_3 receptor (3PBL), the human muscarinic-M_2 receptor (3UON), and the human



Scheme 1. Integrated computational and biological workflow for the prediction of a binding mode of TGR5 agonists.