Rationally designed PPARδ-specific agonists and their therapeutic potential for metabolic syndrome

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The peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors regulates a wide variety of lipid-related genes, including those responsible for adipose differentiation, cholesterol metabolism, and lipid metabolism and transport. There are three different PPAR nuclear receptors, with different localizations and specializations. PPARα is primarily expressed in the liver, heart, kidney, and muscle, and is involved in lipid metabolism, whereas PPARγ is expressed in adipose tissues and is a target for treating type II diabetes because of its role in insulin sensitization (1, 2). The third receptor, PPARδ, is ubiquitously expressed, and activation in animal models improves lipid homeostasis and insulin sensitivity (3). These regulatory roles make the PPAR nuclear receptors attractive targets for treating dyslipidemia and type II diabetes. Fibrates and thiazolidinediones (TZDs) are used to treat these conditions by selectively activating PPARα and PPARγ, respectively. However, there are health issues caused by long-term use of these drugs, and thus a safe compound that specifically targets PPARδ could potentially aid in treatment of both hyperlipidemia and type II diabetes (2, 4). In PNAS, Wu et al. develop a highly selective set of compounds that target PPARδ and discover a mechanism of ligand specificity, which may be unique to the PPAR nuclear receptor family and could aid in further development of highly specific PPAR ligands (5).

The PPAR family of nuclear receptors is implicated in metabolic syndrome, which affects 35% of the United States population and is a major risk factor for developing cardiovascular disease and type II diabetes (6). Metabolic syndrome is defined by a cluster of risk factors, such as obesity, elevated serum triglycerides, dyslipidemia, hypertension, and elevated glucose levels in the fasting state. TZDs activate PPARγ and reduce glucose levels, improve insulin sensitivity, and can improve lipid homeostasis. However, TZD use can increase adipogenesis and lead to increased obesity in patients, and can also increase risk for cardiovascular disease and stroke (4). Because PPARδ regulates lipid transport and uptake, research has been conducted on the efficacy of compounds that act as agonists or partial agonists for both PPARδ and PPARγ, or as pan-agonists for all three PPARs, but no such compounds have successfully gone through trials (7).

The only promising PPARδ agonist thus far is GW501516, which has 1,000-fold specificity for PPARδ over the α- and γ-receptors; its extremely high affinity makes it a strong activator, and binding of the compound induces recruitment of a transcriptional coactivator, PGC-1α (8). In preclinical trials, treatment with this agonist increased lipid metabolism and increased HDL levels, while preventing weight gain on a high-fat diet in mice, and protected animals against developing type II diabetes (8). Despite these promising indications that it could treat metabolic syndrome, treated mice and rats developed cancer and further development ceased (9). It is unclear whether activation of PPARδ or an off-target effect was responsible for carcinogenesis, and a more highly specific ligand is needed to enable researchers to definitively show whether PPARδ activation is a viable therapeutic target, or if activation is too dangerous.

Conservation of Specific Residues in the LBD Allows for Selective Binding to PPARδ

PPAR nuclear receptors share highly conserved ligand-binding domains (LBD), which can make designing receptor-specific ligands difficult. The LBDs are structurally similar, with a conserved activating function-2 (AF-2) region essential for ligand-binding activation. The roughly 1,300 Å³ ligand-binding cavity has three arms: ligands such as lipid head groups bind to the AF-2 region on arm I and extend into the lipophilic arms II and III (10). The size of the cavity directly contributes to the ability of PPAR nuclear receptors to bind a wide variety of natural ligands, including fatty acids and eicosanoids (1). Designed PPAR agonists, such as TZDs and fibrates, take advantage of this Y-shaped cavity by simultaneously occupying both lipophilic arms to further stabilize the receptor in its active conformation (10).

Wu et al. (5) rationally designed ligands to occupy both of these arms of PPARδ, focusing on interacting with residues not shared with the α- and γ-types in a successful attempt to improve the specificity of their compounds. Similar to the GW501516 compound,
their ligands all have a carboxyl head group, which interacts with the AF-2 region, but their ligands are an order-of-magnitude more selective for PPARδ than previous compounds. The authors tested different substituent groups to determine how to best occupy arms II and III while increasing specificity for PPARδ over PPARα and PPARγ. Using this library, Wu et al. determined that several PPARδ-specific residues are essential for the specific binding of their compounds, and the shape of these ligands both determines which PPARδ residues interact with the ligand and causes steric clashes with the larger residues in the binding pockets of PPARα and PPARγ.

Such strong specificity because of a select few residues is supported by prior research. For example, fibrates bind to PPARα but show no activation of PPARδ. Mutating a single conserved methionine residue in the LBD of PPARδ to valine confers the mutant PPARδ with the ability to bind fibrates. PPARα has a valine residue at the equivalent position, and conversely mutating this valine to a methionine residue prevents fibrate binding to the mutant PPARα (11). Multiple groups have also shown that modifying the tail region and any substituent groups on PPAR nuclear receptor pan-agonists greatly changes affinity through introduction or reduction of steric hindrance between the ligand and receptor, indicating that conserved residues are essential for ligand specificity (12). A Unique Trp Residue in a Flexible Loop of PPARδ Aids in Substrate Specificity

While considering structure, it is important to note that the H2′ helix is unique to PPAR nuclear receptors, as is the highly flexible H2′–H3 loop. The loop is so flexible as to be almost disordered in some structures, and is difficult enough to model that it is missing from many available crystal structures of PPAR nuclear receptor LBDs. Through comparing structures of PPARδ bound to conformationally distinct ligands, Wu et al. (5) have found a tryptophan residue in this loop region that dramatically changes conformation upon binding of different ligands. The authors posit that the resulting change in conformation because of the bulkiness of the ligand tail is a mechanism of ligand selectivity by which PPAR nuclear receptors may bind to distinct ligands. The tryptophan residue is not present in PPARα or PPARγ, but is highly conserved in PPARδ. Each PPAR receptor may have other conserved residues in this loop that would further differentiate the subtypes to potential ligands.

Wu et al. (5) have successfully designed a ligand with high selectivity for PPARδ, which will aid researchers in studying the effects of specific PPARδ agonism, and may allay worries about the safety of this approach for treating type II diabetes and dyslipidemia. Perhaps even more importantly, Wu et al. have determined a mechanism by which PPARδ accommodates ligands near the H2′–H3 loop, which is specific to this receptor because of highly conserved residues that it does not share with other PPAR nuclear receptors. This is a potential mechanism for ligand selectivity that could aid in further understanding of PPAR binding mechanisms and future drug design. Understanding of this mechanism could even be used to design ligands that are pan- or partial-agonists that balance activation of PPARs to minimize side effects and maximize treatment of metabolic disease.

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