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論文題目	GPR120 Receptor-Ligand Interaction -Design and Pharmacological Characterization of Novel GPR120 Agonist-		
（論文内容の要旨） GPR120 is a G-protein-coupled receptor (GPCR) whose endogenous ligands have been identified as medium- to long-chain free fatty acids, and is implicated in the secretion of incretin hormones. Despite recent research efforts, little is known about the ligands binding mechanism for GPR120. Moreover, functional studies of GPR120 have been limited by the lack of specific ligands. In my studies, I constructed a homology model of GPR120 that was developed based on a photoactivated model derived from the crystal structure of bovine rhodopsin. I for the first time explored the structure-activity relationships (SARs) of GPR120 agonists by utilizing docking simulation based on the GPR120 homology model, and then identified several novel selective agonists for GPR120. Furthermore, I subjected five putative residues that located in the binding site predicted by the docking simulation to the site-directed mutagenesis analysis. As a result, I identified two residues important for activation of GPR120. Taken together, these studies would be valuable for understanding the molecular mechanism of ligand binding and pharmacological functions of GPR120. Chapter 1. Novel selective ligands for free fatty acid receptors GPR120 To identify selective ligands for GPR120, and for GPR40 that is another receptor whose endogenous ligand is medium- to long-chain free fatty acid, more than eighty natural compounds were screened, together with a reference compound MEDICA16, which is known to activate GPR40, by monitoring the extracellular regulated kinase (ERK) and [Ca²⁺]_i responses in inducible and stable expression cell lines for GPR40 and GPR120, respectively. MEDICA16 selectively activated [Ca²⁺]_i response in GPR40-expressing cells, but not in GPR120-expressing cells. Among the natural compounds tested, grifolin derivatives, grifolic acid and grifolic acid methyl ether promoted ERK and [Ca²⁺]_i responses in GPR120-expressing cells, but not in GPR40-expressing cells, and inhibited the LA-induced ERK and [Ca²⁺]_i responses in GPR120-expressing cells. Interestingly, in accordance with the pharmacological profiles of these compounds, similar profiles of glucagons-like peptide-1 (GLP-1) secretion were seen for mouse enteroendocrine cell line, STC-1 cells, which express GPR120 endogenously. Taken together, these studies identified novel selective agonists for GPR120 and GPR40. Chapter 2. Structure-activity relationships of GPR120 agonists based on a docking simulation			

I have identified several natural compounds as selective GPR120 partial agonists described as chapter 1. In order to further develop GPR120-specific agonists, a series of compounds (denoted as NCG compounds) derived from a PPAR γ agonist was synthesized and their structure-activity relationships (SARs) as GPR120 agonists were explored in this study. To examine the agonistic activities of these newly synthesized NCG compounds, and of compounds already shown to have GPR120 agonistic activity (grifolic acid and MEDICA16), I conducted docking simulation in GPR120 homology model that was developed based on a photoactivated model derived from the crystal structure of bovine rhodopsin. I calculated the hydrogen bonding energies between the compounds and the GPR120 model. These energies correlated well with the GPR120 agonistic activity of the compounds ($R^2 = 0.73$). NCG21, the NCG compound with the lowest calculated hydrogen bonding energy, showed the most potent ERK activation in a cloned GPR120 system. Furthermore, NCG21 potently activated ERK, intracellular calcium ($[Ca^{2+}]_i$) responses and GLP-1 secretion in murine enteroendocrine STC-1 cells that express GPR120 endogenously. Moreover, administration of NCG21 into the mouse colon caused an increase in plasma GLP-1 levels, a finding consistent with the result of *in vitro* assays. Taken together, these data demonstrate that the docking simulation utilizing a homology-based model successfully predicted the agonistic activity of compounds.

Chapter 3. Ligand recognition mechanism of GPR120

Despite the important physiological implications of GPR120, much still remains unclear in our understanding of the actual mechanism of ligand binding. In this study, I sought to understand the molecular basis of ligand recognition and to identify the residues involved in ligand binding in GPR120. I constructed molecular model of GPR120 and estimated the residues, which may contribute to the binding of ligands. These predicted residues were subjected to site-direct mutagenesis analysis. α -linolenic acid (α -LA) known to activate GPR120 promoted ERK and $[Ca^{2+}]_i$ activity in cells expressing GPR120 wild type (WT), R15A, H111A, R177A and R182A, but not in R99A. On the other hand, Thiazolidinedione troglitazone that have no carboxylic acid in the structure promoted ERK and $[Ca^{2+}]_i$ activity in all of these mutants except for R15A. Moreover, flow cytometer-based binding assay showed that specific fluorescent-labeled FFA C8-BODIPY-C5 bind to GPR120 in saturable manner with an apparent dissociation constant of $\sim 2 \mu M$. Interestingly, the level of binding of C8-BODIPY-C5 was significantly lower in GPR120 R99A than in WT. Therefore, in this study, I demonstrated that the Arg15 and Arg99 are important for the activation of GPR120, and Arg99 particularly contributed to interaction of carboxylic acid of FFA ligands.

In conclusion, I characterized the molecular basis of ligand recognition and activation of GPR120 by using SARs and pharmacological studies. In addition, I identified several natural and synthesized GPR120 ligands, which would be valuable probes to further investigate the physiological functions of GPR120.

(続紙 2)

(論文審査の結果の要旨)

本論文は、脂肪酸受容体GPR120について、特異的リガンドの探索と相互作用解析を目的として、強制発現細胞系を用いたリガンド候補化合物群の活性評価と、GPR120受容体ホモロジーモデルを用いたドッキングシミュレーションにより結合様式の解析を行った論文である。

中・長鎖脂肪酸によって活性化されるGPR120受容体は、腸管・脂肪組織・肺に発現し、インクレチンホルモンの分泌に関与していることが報告されていることから、糖尿病を始めとした各種代謝疾患の創薬ターゲットとして注目されている。GPR120は、世界的にも精力的に研究が行われているが、特異的リガンドに関する報告が僅かであること、また既知リガンドについても結合様式に関する情報が殆どないこと等から、生理学的な機能解析が十分に進んでいないのが現状である。そこで申請者は、天然物化合物および合成化合物群から新規GPR120リガンドの探索を行い、さらにこれらの化合物とGPR120受容体ホモロジーモデルによるドッキングシミュレーション、および変異受容体株による検討から、その結合様式について詳細な検討を行った。

申請者は、GPR120受容体の強制発現細胞株を用いて、80種以上の天然物化合物とその類縁体から、細胞内Ca²⁺濃度およびERK活性化を指標としてスクリーニングを行い、新規GPR120リガンドを探索した。その結果、grifolin類縁体のうち、grifolic acidとgrifolic acid methyl etherの2種の化合物に活性が認められた。さらに、内在性にGPR120を発現する腸管内分泌細胞株による検討から、これらの化合物がいずれもグルカゴン様ペプチドの分泌を促進することが明らかとなった。

次に、より活性の高い化合物の探索を目的として、PPAR γ リガンドを基に一連の化合物群(NCG化合物)を合成し、ERK活性化能とドッキングシミュレーションによる水素結合エネルギーを評価したところ、両者には相関が認められた ($R^2 = 0.73$)。特に、NCG化合物の中でNCG21が最も高いERK活性能を示し、かつ最も低い水素結合エネルギーを示したことは、本評価手法が新規リガンドの評価系として非常に有用であることを示唆している。また、変異受容体株による検討からは、99番目のArg残基が、NCG21及び内因性リガンドである α -リノレン酸が有するカルボキシル基との相互作用に関与し、GPR120の活性化を引き起こしていることが明らかとなった。本結果は、ドッキングシミュレーションから予測される結合様式とも一致していたことから、構築したGPR120受容体ホモロジーモデルが化合物の活性予測だけでなく、相互作用の理解にも非常に有用であることが示唆された。

本論文で申請者は、脂肪酸受容体GPR120について、新規リガンドの探索とリガンド結合様式を検討することにより、リガンドデザインに有益な構造情報とその評価手法を得ることに初めて成功した。これらの結果はGPR120のみならず脂肪酸受容体研究全般の更なる進展に寄与するものであり非常に重要な知見となると考えられる。

よって本論文は博士(薬科学)の学位論文として価値あるものと認める。さらに、平成22年8月9日論文内容とそれに関連した口頭試問を行った結果、合格と認めた。

論文内容の要旨及び審査の結果の要旨は、本学情報リポジトリに掲載し、公表とする。特許申請、雑誌掲載等の関係により、学位授与後即日公表することに支障がある場合は、以下に公表可能とする日付を記入すること。

要旨公開可能日： 平成 年 月 日以降