（学位第３号様式）

学 位 論 文 要 旨

氏　名	Tomotaka Nagasawa
題　目	The novel non-nutritive food functions via FFAR4/incretin signaling （FFAR4 ／インクレチンシグナリングに関わる非栄養性食品分子の新規機能の解明）

Metabolic syndrome refers to conditions in which hypertension, hyperglycemia, and dyslipidemia with excessive accumulation of triglycerides. It is considered in combination to increase the risk of diabetes, arteriosclerosis, and liver cirrhosis. The number of metabolic syndrome patients is increasing year by year due to the westernization of eating habits. Therefore, the development of the treatment / prevention method is very important and is a social urgent need.

FFAR4 / GPR120 is a receptor for long-chain fatty acids and belongs to the G protein-coupled receptor (GPCR). It is mainly expressed in small intestinal endocrine L cells and induces GLP-1 secretion. GLP-1, which is one of incretins, is known to act as anti-metabolic syndrome. In recent studies, FFAR4 is a potential target of pharmaceuticals for type II diabetes. From this, control of incretin signaling is one of the keys to control of metabolic syndrome. In this study, we examined whether food ingredients possibility control incretin signaling, and elucidate the food function that has a new mechanism for anti-metabolic syndrome.

In this thesis, I have performed three major studies. That is, 1. Development of new FFAR4 ligand screening method, 2. Search for new FFAR4 ligand, and 3. Identification of binding site between FFAR4 / ligand. In the first study, I have established the assay method to detect FFAR4 activation by ligands quantitatively and with high sensitivity. The TGFα shedding assay was originally developed by Inoue et al. as a method for ligand screening for orphan GPCRs. I applied this assay to FFAR4. In the second study, we found that phytosphingosine (PHS) and teadenol A are novel ligand for FFAR4. PHS is found in the plasma membranes of yeast and dietary PHS can be incorporated by consuming bread and fermented foods. Teadenol A is a polyphenol recently isolated Japanese post-fermented tea. Many of the natural ligands of FFAR4 have a carboxyl group, and it has been thought that the carboxyl group plays an important role in the ligand recognition of FFAR4. However, PHS does not have a carboxyl group, raising the question of how PHS recognizes FFAR4. Finally, in the third study, we performed docking simulation analysis using a FFAR4 homology model to predict binding sites between FFAR4 and ligands. The docking simulation revealed that the probable hydrogen bonds to FFAR4 differ between PHS and α-linolenic acid, known as a FFAR4 ligand. Furthermore, amino acid mutants of the expected binding sites were prepared and analyzed in detail. I found that PHS binds to glutamic acid at position 249 of FFAR4 and α-linolenic acid binds to arginine at position 264. This study revealed that PHS activates FFAR4 in a different binding manner than previously known ligands.

The ligand of FFAR4 has been considered to be a free fatty acid, but this study showed for the first time that PHS and teadenol A, which are also contained in foods, could be ligands.