論文の内容の要旨

論文題目 Anti-diabetic effects of phytochemicals
and analysis of their modes of actions
（ファイトケミカルの抗糖尿病効果とその作用機構解析）

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Many active components derived from natural resources such as plant extracts have recently attracted the attention of scientific world for their potential use as drugs in treating metabolic diseases such as diabetes. In this study, I investigated the effect of two phytochemicals, genistein and nepodin, on type 2 diabetes and analyzed their modes of actions. Genistein, an isoflavone of soybean components, was studied with collaborators at Tokyo Noko University. Nepodin, acetyl-1, 8-dihydroxy-3-methyl naphthalene, is a component found from the root of *Rumex japonicus,* a common perennial herb that grows in East Asia. It has traditionally been used by the local people in Okinawa, Japan for treatment of acute and chronic cutaneous diseases, and by the Chinese as an effective drug for the treatment of constipation, jaundice, uterine hemorrhage, and hematemesis. I identified nepodin, a small molecular activator of glucose uptake in cultured L6 myotubes through high throughput screening of small compound library consisting of 2400 compounds.

As for the effect of nepodin on type 2 diabetes *in vivo*, I used male C57BL/KsJ-*db*/*db* mice (5 weeks of age), a genetically obese diabetic mouse strain, and C57BL/KsJ-*m*/*m* mice as nondiabetic lean group, to investigate the regulatory mechanism of nepodin on glucose homeostasis. During 5 weeks of oral nepodin administration (2 mg/kg/day and 10 mg/kg/day), I found that nepodin attenuates the increase of fasting blood glucose level dose-dependently without changes of food intake and weight in *db*/*db* mice. I conducted an intraperitoneal glucose tolerance test (IPGTT) to examine the effect of nepodin on the glucose tolerance. I found that nepodin improves the impaired glucose tolerance of C57BL/KsJ-*db*/*db* mice. Moreover, I found that nepodin decreases lipid levels such

as triglyceride and cholesterol in serum and liver and inhibits plasma concentrations of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation and oxidative stress in *db*/*db* mice. In addition, I found that in quantitative RT-PCR assay, nepodin inhibits the expression of genes related to fatty acid synthesis, including fatty acid synthase (FAS) and stearoyl-Coenzyme A desaturase 1 (SCD1), while increasing the expression of glycogen synthase (GS) in the liver of *db*/*db* mice. To confirm direct effect of nepodin on gene expression associated with glucose homeostasis and lipid metabolism, I performed quantitative RT-PCR assay using C_2C_{12}, a mouse skeletal muscle cell line. I found that nepodin increases the expression of GLUT4 and genes related to fatty acids oxidation such as carnitine palmitoyltransferase I (CPT1α), Pyruvate dehydrogenase kinase (PDK1) and medium-chain acyl-CoA dehydrogenase (MCAD).

In vitro, I investigated the effect of genistein and nepodin on glucose uptake and the mechanism of glucose uptake enhancement by cultured L6 myotubes under both normal glucose (5.5 mM) and high glucose (25 mM) conditions, mimicking the normoglycemic condition and the hyperglycemic condition in diabetes, respectively. I found that both genistein and nepodin significantly stimulate glucose uptake in differentiated L6 myotubes under both glucose conditions. Through western blotting of GLUT4 in plasma membrane fraction by subcellular fractionation in L6 myotubes, and via the immunocytochemistry of GLUT4 in L6 myoblast transfected with pFC21A-Glut4-Halo, I found that both genistein and nepodin stimulated the translocation of GLUT4, a main glucose transporter responding to insulin signaling, to plasma membrane in differentiated L6 myotubes under both glucose conditions. As for nepodin, I found robust immunofluorescent signals for Glut4-Halo. Through further study using inhibitors of kinases related to glucose uptake in skeletal muscle cells, I found that the stimulatory effect of genistein on glucose uptake was be dependent on the PI3-kinase, mTOR, PKC and AMPK pathway, and that the stimulatory effect of nepodin on glucose uptake was dependent on the AMPK and Sirt1pathways under both glucose conditions. Moreover, I found that specifically both genistein and nepodin stimulate the phosphorylation of AMPK time-dependently under both glucose conditions. In skeletal muscle of *db*/*db* mice, nepodin rescued the impaired phosphorylation of AMPK.

In summary, these results suggest that both genistein and nepodin have antihyperglycemic potentials, including inhibition of the increase of fasting blood glucose and improvement of impaired glucose tolerance in *db*/*db* mice, and that this effect is mediated through the regulation of gene expression related to glucose homeostasis by a mechanism including both the activation of AMPK and GLUT4 translocation.