의학석사 학위논문

Regulation of sortilin expression in skeletal muscle

근육 Sortilin의 발현조절기전

2017년 8월

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Abstract

Sortilin is a multiligand receptor, and belongs to the family of Vps10p domain receptors. Sortilin mainly localizes in the trans-Golgi network and a small amount of sortilin also localizes in the plasma membrane, where sortilin regulates intracellular trafficking, secretion and endocytosis of target proteins. A number of recent studies demonstrated that hepatic sortilin directed ApoB for lysosomal degradation, inhibited ApoB100 secretion, and increased LDL uptake and catabolism, suggesting that hepatic sortilin is involved in lipid metabolism. However, the role of sortilin in skeletal muscle remains unclear.

In this study, I investigated expression levels of sortilin in several tissues from diabetic mice. I found that sortilin protein levels in skeletal muscle were increased in high fat diet (HFD)-induced obese mice and db/db mice, but sortilin levels in liver and adipose tissues were decreased in the diabetic mice.

To figure out what factors regulate sortilin protein levels in diabetic conditions, I tested the effects of several hormones, fatty acids, and cytokines on sortilin protein levels in C2C12 myotubes. Interestingly, sortilin protein was significantly

decreased when C2C12 myotubes were maintained in the low level of glucose. The mRNA level of sortilin was not changed by glucose levels, and the stability of sortilin protein was decreased under the low level of glucose. The effect of glucose levels on sortilin stability was not observed in the presence of proteasome inhibitor, suggesting that the low level glucose condition facilitates proteasome-mediated degradation of sortilin. Also, when I determined a physiological role of sortilin according to the glucose levels, sortilin was involved in glucose uptake by insulin stimulated Glut4 translocation in L6-GLUT4myc myotubes.

Keywords : Sortilin, trafficking, Glut4,glucose uptake, lysosome degradation, obesity, Type 2 diabetes

Student Number : 2015-26085

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Introduction

Sortilin is identified as a neurotensin receptor in nervous systems, and a member of the vacuolar protein sorting 10 protein (Vps10p) domain family, which is produced as a precursor protein and then activated by removal of the N-terminal propeptide in late Golgi compartments. Sortilin mainly localizes in the trans-Golgi network (TGN), and is involved in sorting target proteins for secretion and endosomal transport. A small amount of sortilin localizes in the cell membrane, and regulates receptor-mediated endocytic uptake [1]

Sortilin is especially involved in trafficking pathway related to lipid metabolism. Hyperglycemia in diabetes promotes cardiovascular disease, which is mediated by changes of lipoprotein metabolism. The lipoprotein metabolism affects the intercellular transport of cholesterol and triglycerides. Apolipoprotein B is involved in the development of diabetes-induced cardiovascular disease through integration of chlyomicrons, very low density lipoprotein(VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL).[2] Sortilin plays an important role in ApoB-VLDL secretion in

liver. Sortilin not only inhibits ApoB secretion but also facilitates degradation of ApoB-VLDL complex. Liver specific sortilin overexpression inhibits VLDL secretion and consequently decreases serum lipid levels in obese and diabetic mice.[3] However, sortilin whole-body knockout improves metabolic phenotypes: body weight and hepatic TG accumulation decrease in sortilin whole-body knockout mice comparing to WT mice. In addition, Sortilin whole-body knockout significantly improves insulin tolerance and reduces development of insulin resistance in diet-induced obesity (DIO) condition, along with increased insulin signaling in liver and adipocyte tissue.[4]

Skeletal muscle is the primary tissue responsible for insulin-stimulated glucose disposal, thus inability of skeletal muscle to uptake glucose in an insulin dependent manner is main cause of insulin resistance which is characteristic of Type 2 diabetes.[5] In skeletal muscle, expression and translocation of two kinds of glucose transporter, Glut1 and Glut4, are regulated during myocyte differentiation. Particularly in the case of Glut4, its expression and translocation to the cell surface upon insulin stimulation are increased during myocyte differentiation.[6] Thus the increased cellular Glut4 content contributes to

development of the insulin-regulated glucose transport system and improvement of insulin sensitivity.[5] Sortilin is known to involve Glut4 translocation which is stimulated by insulin signaling. Also, sortilin is predominantly expressed in adipocyte and located in vesicles containing Glut4, and translocated to the cell membrane in response to insulin.[7-11] Moreover, sortilin overexpression, in vitro, increases glucose uptake and Glut4 translocation under insulin stimulation. [12, 13]

L6 cells stably overexpressing Glut4-myc (L6-GLUT4myc) have been generated, which have extracellular myc-tagged Glut4 when Glut4 is translocated to the plasma membrane. Consequently, insulin-stimulated Glut4 translocation in L6-GLUT4myc myotubes can be easily detected with Glut4, myc antibodies.[14-17]

Our laboratory has found that muscles from diabetes patients have high levels of sortilin. Therefore, in this study, I tried to understand the mechanism by which sortilin expression is regulated and the functional roles of sortilin in skeletal muscle under diabetic condition.

Material and Method

Materials

Cycloheximide and MG132 were from Sigma (St Louis, MO, USA).

Anti-Sortilin antibody was purchased from Alomone Labs (Jerusalem, Israel) and R&D Systems (Minneapolis, MN, USA). Anti-γ tubulin antibody was purchased from Sigma (St Louis, MO, USA). Anti-myc antibody and anti-Glut4 antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). sisortilin and nonspecific siRNA (siNS) were obtained from Dharmacon, Inc. (Chicago, IL,USA)

Animals

Animal studies were performed in accordance with the Institutional Animal Care and Use Committee of Seoul National University Hospital. For a control diet group, 5-week old C57BL/6 mice were fed chow diet (Cargil Agri Purina, Gangnam-gu, Seoul, Korea), and for a diet-induced obese

(DIO) group, mice were fed high fat/high sucrose diet (58 Kcal% fat with sucrose Surwit Diet) (D12331, Research Diets inc., New Brunswick, NJ) for 12 weeks or 25 weeks. C57BLKS/J-*db/db* and lean control (db/m) mice were purchased from SLC (SLC, Shizuoka, Japan) and sacrificed at 13 weeks of age.

For tissue analysis, mice were anesthetized with Avertin (500mg/kg). All efforts were made to minimize pain during sacrifice. Tissues were isolated, snap-frozen in liquid nitrogen, and stored at- 80℃ until use.

Cell culture, differentiation and transfection

Mouse skeletal muscle cell line, C2C12 myoblasts were maintained in High glucose (25mM) Dulbecco's Modified Eagle Medium (DMEM) (Hyclone laboratories, Logan, UT) supplemented with 10% FBS (invitrogen, Carlesbad, CA) at 37℃ under a 5% CO2. Differentiation was induced using DMEM with 2% horse serum (Invitrogen, MA, USA), and the media were changed every two days. Five days after 2% horse serum addition, C2C12 myotubes were harvested for RNA and protein preparation. For low glucose treatment, C2C12 myotubes were incubated in Low glucose (5mM) DMEM (Welgene, Korea) at

day 5 of differentiation. L6-GLUT4myc cells stably myc-tagged GLUT4myc were described previously.[15, 16] L6-GLUT4myc myoblasts and L6-GLUT4myc myoblasts grown on 0.1% gelatin coated coverslips were maintained in High glucose DMEM supplemented with 10% FBS. Differentiation was induced using DMEM with 2% horse serum. Five days after 2% horse serum addition, L6-GLUT4myc myotubes were transfected with siRNA (50nM) using Lipofectamine RNAiMAX (Invitrogen, MA, USA) for 48 hours.

RNA preparation and Real-time PCR

Total RNAs from differentiated C2C12 cells were isolated using TRIzol reagent (Invitrogen, MA, USA) according to the manufacturer's instruction. To synthesize cDNA, 1μg of total RNA, 10μl of reaction buffer, 5μl of 100mM DTT, 2.5μl of 10mM dNTP, 1μl of Oligo dT, 0.5μl of RNase inhibitor and 2μl of RTase(Invitrogen, MA, USA) were mixed and RNA free water added up to 20μl. The mixture was incubated at 37℃ for 1 hour and at 72℃ for 10 min using PCR system. Expression levels of genes were determined by using quantitative real-time PCR with SYBR-MASTER MIX (Takara, Otsu, Shiga,

Japan) and ABI 7500 Real-time PCR system (Applied Biosystem, CA, USA). The primer sequences for qPCR were listed on Table 1.

Western blot analysis

Cells were harvested in RIPA buffer (0.5M Tris-HCl, pH 7.4, 1.5M NaCl, 2.5% deoxycholic acid, 10% NP-40, 10mM EDTA) (Merck Millipore, Temecula, CA) containing protease inhibitors (10 μg/μl aprotnin, 10 μg/μl leupeptin and 1mM PMSF). Cell debris were removed by centrifugation (13,000 rpm) for 20 min at 4 ℃.

Frozen mouse tissues were powdered in liquid nitrogen and incubated in RIPA buffer on the rotator for 2 hours at 4 ℃. Concentration of protein was determined by bicinchoninic-acid (BCA) assay. About 15~20μg of proteins were separated on SDS-PAGE and transferred to a nitrocellulose membrane (Whatman, Dassel, Germany). Membranes were incubated in blocking solution (5% skim milk in Tris-buffered saline with Tween 20) for 1 hour at room temperature and then incubated with primary antibody overnight at 4℃ or 3 hour at room temperature. The proteins bands were detected using the

following antibodies: anti-Sortilin , anti- γ tubulin, anti-Glut4.

Table 1. Primer sequences used for quantitative PCR

(species: *Mus musculus*)

	Forward primers	Reverse primers
Sortillin	5′ CTT TCA TTA CCC GCC AGT GG 3′	5′ GGA TGA CTT CCG TAG CCG TA 3′
GAPDH	5′ AGG TCG GTG TGA ACG GAT TTG 3′	5′ TGT AGA CCA TGT AGT TGA GGT CA 3′

Glucose uptake assay

C2C12 myocblasts were cultured in 24 well plates, and differentiated into myotubes for 7days. After 3 h serum starvation in DMEM, C2C12 myotubes were incubated with KRPH buffer (20mM HEPES, 5mM KH_2PO_4, 1mM $MgSO_4$, 1mM $CaCl_2$, 136mM NaCl, 4.7mM KCl, pH7.4) with 2% bovine serum albumin (BSA) for 40 min. And then, cells were stimulated with or without 1μM insulin for 20 min in presence of 10mM 2-DG. Glucose uptake was measured using a Glucose uptake colorimetric assay kit (ab136955, Abcam Inc. Cambridge, USA) according to the manufacturer's protocols.

GLUT4myc translocation

L6-GLUT4myc myotubes were differentiated on glass coverslips and treated with or without 100nM insulin for 30min after serum deprivation for 3 hours. Myotubes were fixed with 4% formaldehyde in PBS at room temperature for 10min. Cells were incubated with 5% normal goat serum in 0.05% PBST at room temperature for 1 hour. Myc was stained with primary polyclonal anti-Myc antibody and Alexa-568 anti-mouse conjugate combination. Fluorescence images obtained with fluorescent microscope Nikon Y-TV55 (Nikon, Japan).

Statistical Analysis

Statistical analysis of data was used for GraphPad Prism version 5.0(GraphPad Software, Inc., La Jolla, CA). Results are expressed as the mean ± SEM. Statistical significance was calculated using the Mann-Whitney U-test. A p-value less than 0.05 was denoted as statistically significant difference.

Results

I. Expression of sortilin in mouse tissues.

Expression of sortilin in skeletal muscle, Liver, and white adipose tissues from db/m or db/db mice.

The diabetic mice, *db/db* mice, and *db/m* mice were housed for 13 weeks and their gastrocnemius muscle, liver and white adipose tissues were subjected to protein and mRNA extraction. Sortilin protein level was increased by 5 times in gastrocnemius muscle (GM) of *db/db* mice comparing to the muscle of *db/m* mice, the control group (Fig. 1A). On the other hand, sortilin protein level in liver was significantly decreased in *db/db* mice (Fig. 1B). sortilin mRNA levels in gastrocnemius muscle were slightly increased while sortilin mRNA levels in liver and white adipose tissue were decreased in *db/db* mice (Fig. 2).

Expression of sortilin in the DIO (diet induced obesity) mouse skeletal muscle, Liver, and white adipose tissue.

Next, I determined the sortilin expression in diet induced diabetic mice. C57BL/6 male mice were fed with control diet (CD) or with high fat diet (HFD) for 15 weeks, 25 weeks. Not only body weight but also fasting glucose levels were increased in the DIO mice (Fig. 3).

Lysates (15ug) of various tissues were subjected to immunoblot analysis using an anti-sortilin antibody. The sortilin levels in GM of HFD mice were higher than that of WT mice. On the other hand, sortilin levels in liver and WAT were lower in HFD mice (Fig. 4). Sortilin mRNA levels in GM of DIO mice were similar to those of WT mice while sortilin mRNA levels were decreased in liver and WAT after 25 week-HFD feeding (Fig. 5). These results suggest that sortilin expression is differently regulated in GM versus in liver or adipose tissue. In addition, sortilin protein levels are increased in GM of diabetic mice without significant increase of the mRNA levels.

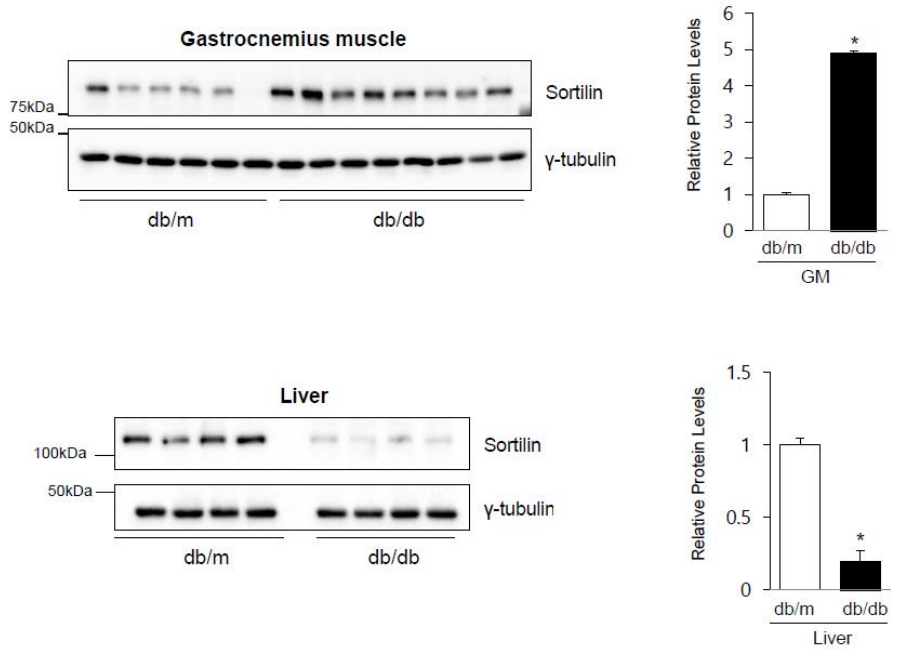


Figure 1. Protein levels of sortilin in skeletal muscle, and liver tissues from db/db mice.

Lysates(15ug) of gastrocnemius muscle(GM) and liver in db/m and db/db mice were subjected to immunoblot analysis using an anti-Sortilin. db/m GM (n=6), db/db GM (n=8), db/m Liver (n=4), db/db Liver (n=4). (* = p <0.05 vs db/m)

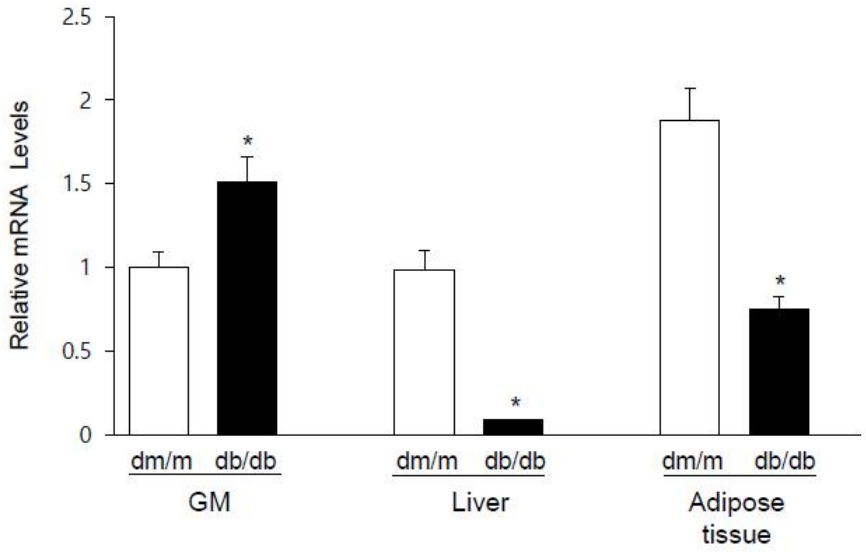


Figure 2. Sortilin mRNA levels in db/db mouse skeletal muscle, liver and white adipose tissue.

Total RNA was isolated from mouse gastrocnemius muscle, liver and epididymal fat. The RNA(1ug) was subjected to real-time PCR with primers specific to sortilin (Table1). db/m GM (n=6), db/db GM (n=10), db/m Liver (n=4), db/db Liver (n=4), db/m WAT (n=4), db/db (n=4). (* = p <0.05 vs db/m)

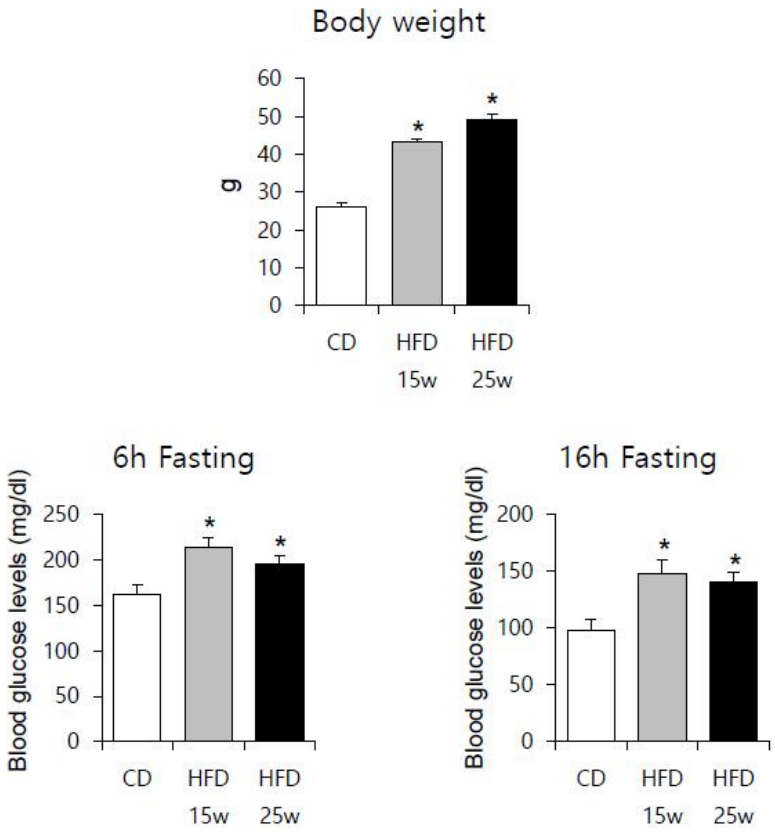


Figure 3. Blood glucose levels and body weight of CD or HFD mice.

C57BL/6 male mice were fed with Control diet (CD, n=6) or with high fat diet for 15 weeks (HFD, n=6) or 25 weeks (HFD, n=9). Their body weights were measured before sacrifice. For Blood glucose levels, mice were fasted for 6h or 16h and then blood was harvested from the mouse tails. (* = p <0.05 vs CD)

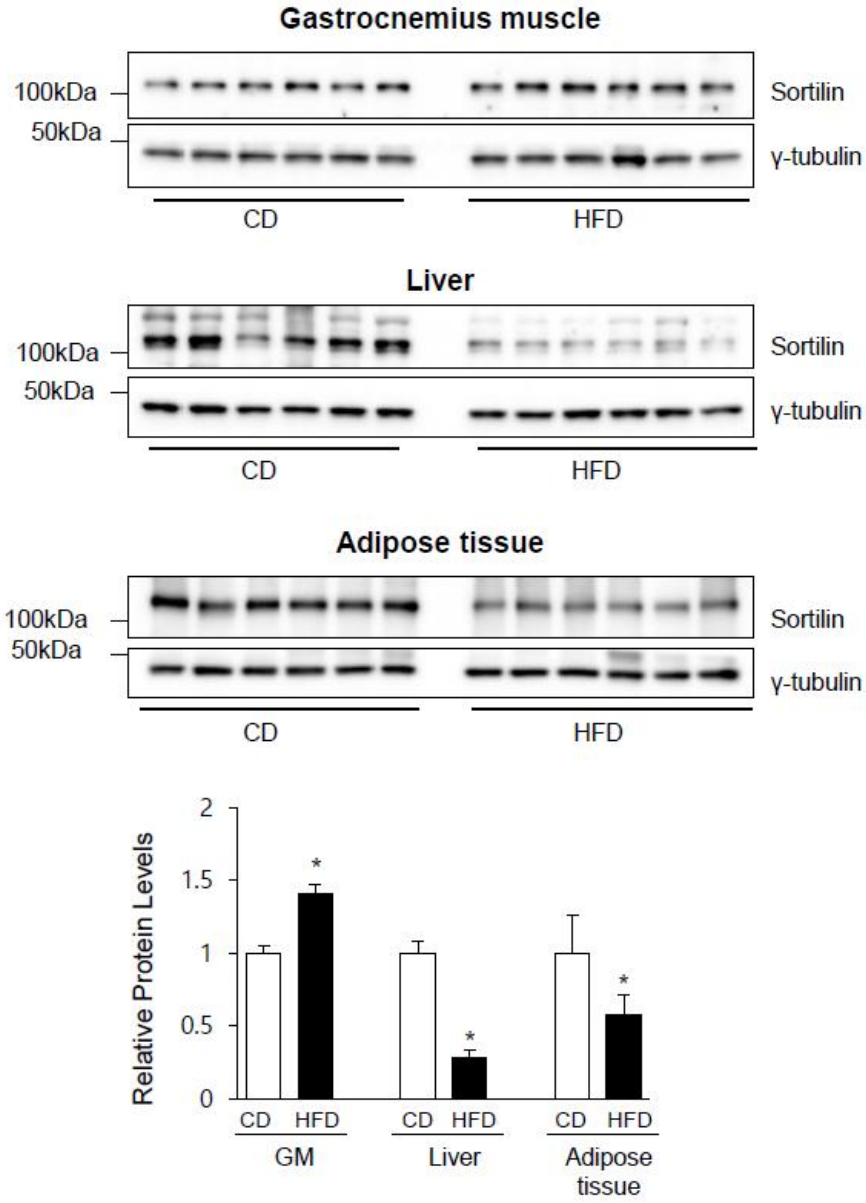

Gastrocnemius muscle
100kDa
50kDa
Sortilin
γ-tubulin
CD
HFD
Liver
100kDa
50kDa
Sortilin
γ-tubulin
CD
HFD
Adipose tissue
100kDa
50kDa
Sortilin
γ-tubulin
CD
HFD
Relative Protein Levels
2
1.5
1
0.5
0
*
*
*
CD HFD
CD HFD
CD HFD
GM
Liver
Adipose tissue

Figure 4. Sortilin protein levels in DIO mouse skeletal muscle, liver, and white adipose tissue.

Protein extracts were prepared from GM, liver and epididymal fat of CD and HFD mice. The intensity of each sortilin band was normalized to that of γ tubulin. (* = p <0.05 vs CD)

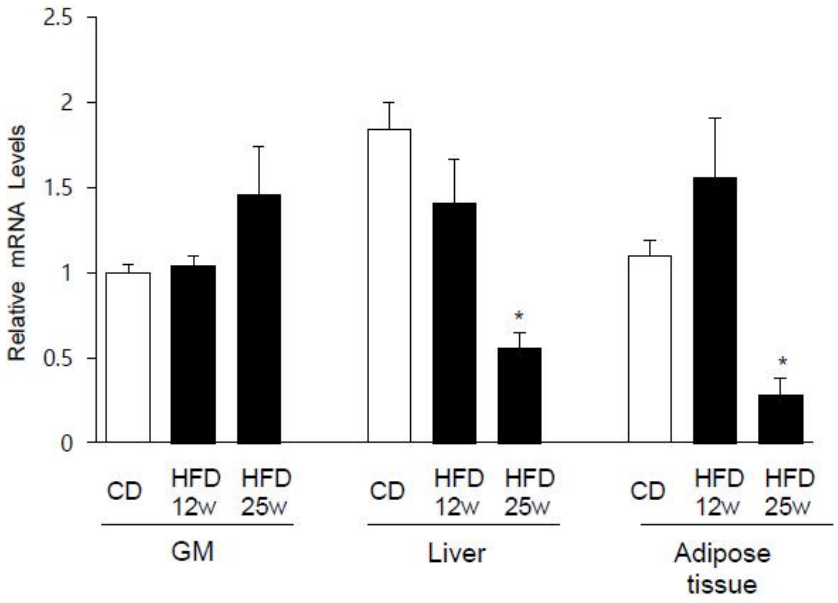


Figure 5. Sortilin mRNA levels in DIO mouse skeletal muscle, liver, and white adipose tissue.

Total RNA was isolated from mouse gastrocnemius muscle, liver and epididymal fat. The RNA(1ug) subjected to real-time PCR with primers designed for specific sortilin. CD GM, liver and epididymal fat (n=5), HFD 12 weeks GM, liver and epididymal fat (n=6), HFD 25 weeks GM, liver and epididymal fat (n=9), (* = p <0.05 vs CD)

II. Identification of a regulator of sortilin expression in C2C12 myotubes.

Next, I tried to find out the factors regulating sortilin protein levels in skeletal muscle. C2C12 myotubes were treated with several hormones or fatty acids, etc. The sortilin protein levels were not changed upon treatment with leptin, rosiglitazone, metformin, interleukin-6(IL-6) or insulin (Figure 6). In addition, palmitate treatment did not affect sortilin protein levels in various doses or time conditions (Figure 7). However, sortilin was markedly decreased by treatment with low glucose (5mM) and the decrease of sortilin occurred in a time dependent manner. In contrast, the mRNA levels of sortilin were not changed by glucose levels (Figure 8 and 9). In addition, the sortilin levels decreased under low glucose condition was reversed by treatment of high concentration of glucose (Figure 8).

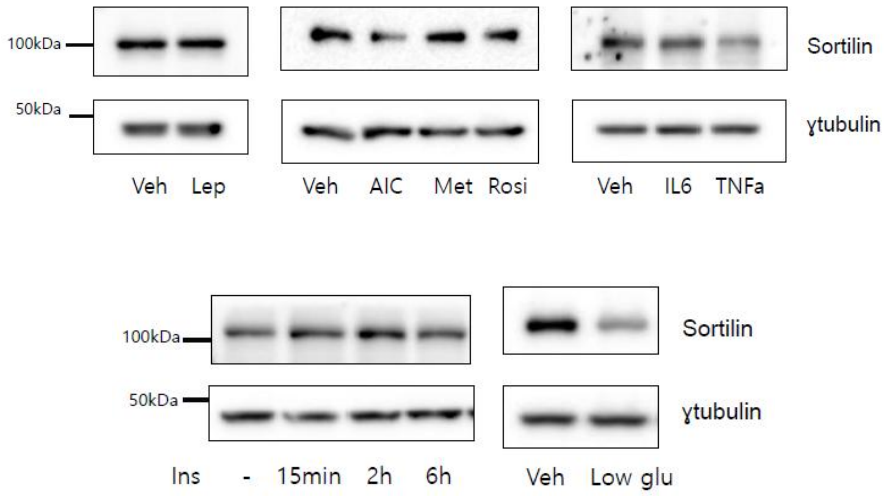
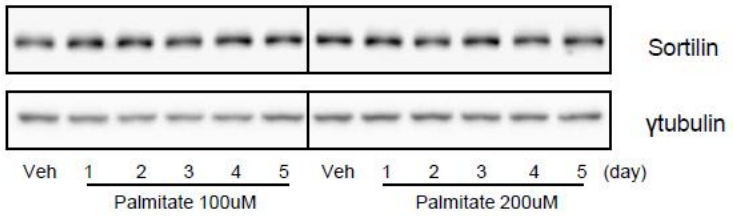
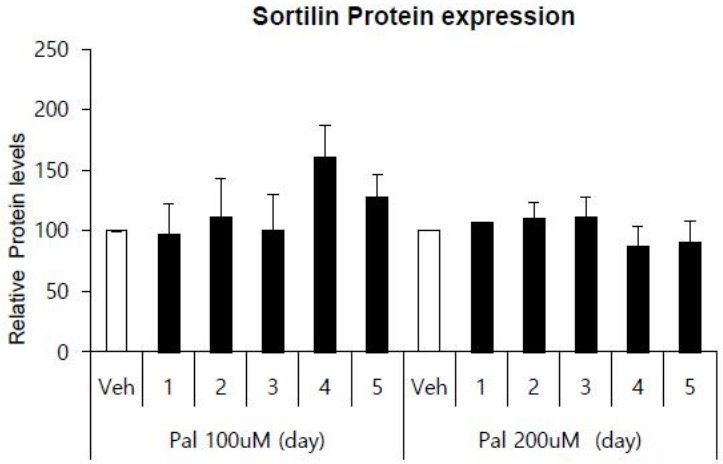
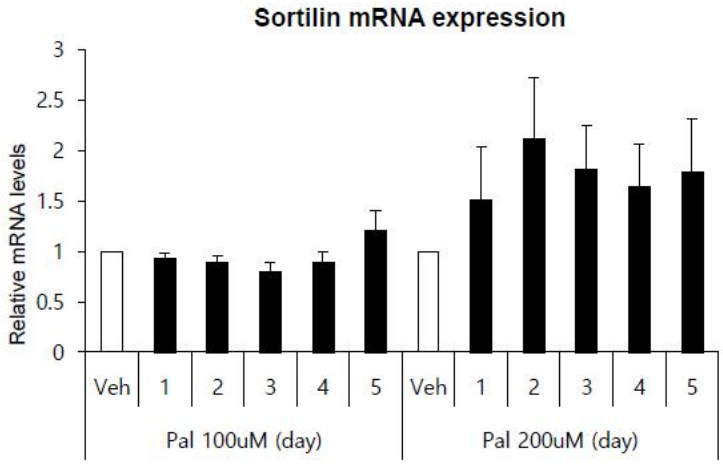


Figure 6. Screening of factors regulating sortilin expression in C2C12 myotubes.

C2C12 myotubes were fully differentiated in high glucose (25mM) DMEM with 2% horse serum. C2C12 myotubes were treated with Leptin (100ng/ml), IL-6 (50ng/ml), TNFa (50ng/ml), AICAR (0.5mM), Metformin (1mM), Rosiglitazone (10uM), or low glucose (5mM) for 24h, or insulin (100nM) for the indicated periods. Cell lysates were subjected to immunoblot analysis.


Sortilin
γtubulin
Veh 1 2 3 4 5 Veh 1 2 3 4 5 (day)
Palmitate 100uM
Palmitate 200uM


Sortilin Protein expression
Relative Protein levels
250
200
150
100
50
0
Veh 1 2 3 4 5 Veh 1 2 3 4 5
Pal 100uM (day)
Pal 200uM (day)


Sortilin mRNA expression
Relative mRNA levels
3
2.5
2
1.5
1
0.5
0
Veh 1 2 3 4 5 Veh 1 2 3 4 5
Pal 100uM (day)
Pal 200uM (day)

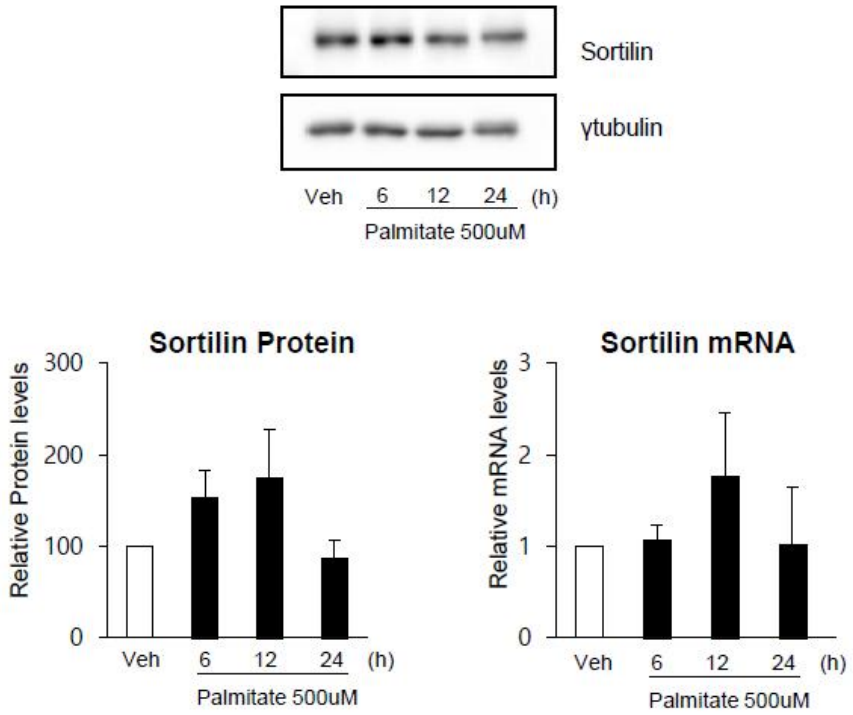


Figure 7. Palmitate has no effect on sortilin mRNA and protein.

C2C12 myotubes were treated with palmitate (100uM, 200uM, or 500uM) in the indicated time course. No statistically significant change was observed. n=3.

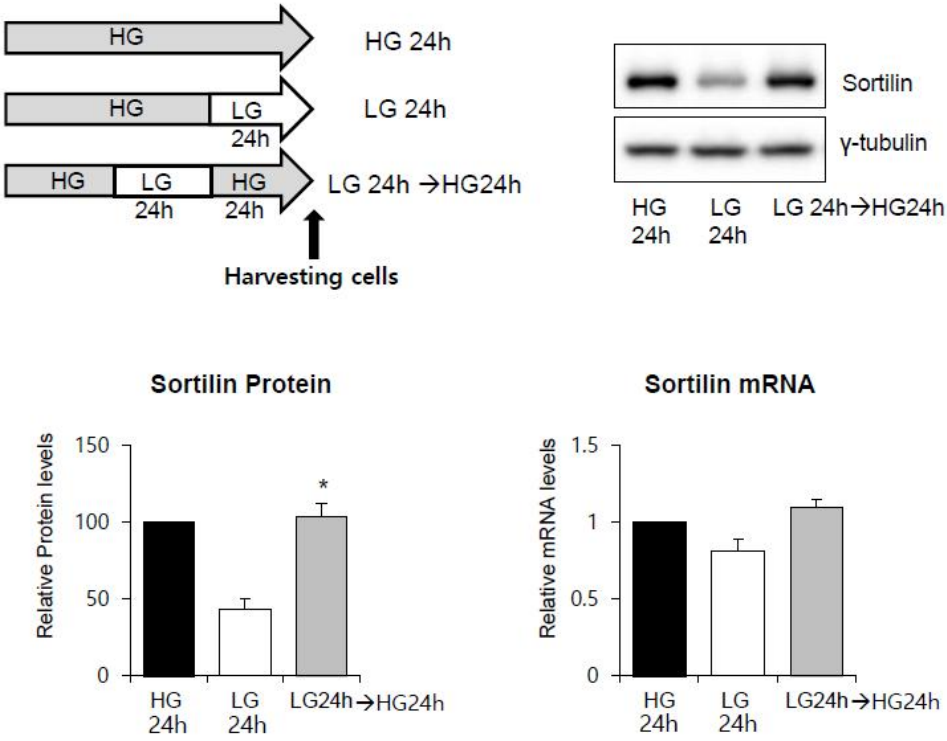
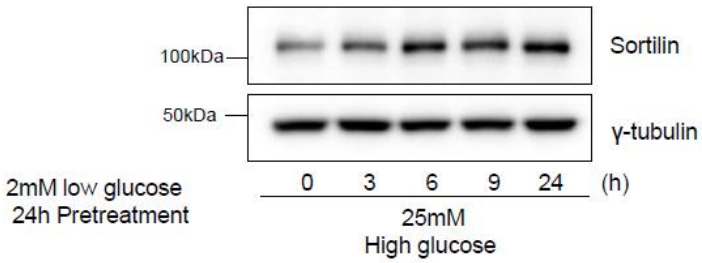
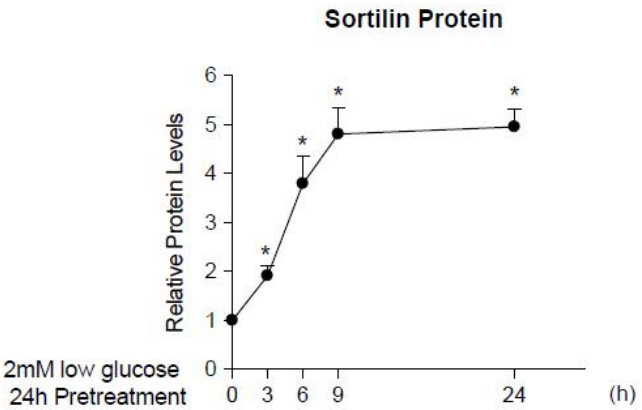


Figure 8. Glucose concentration in the media regulates protein levels of sortilin in C2C12 myotubes.

Fully differentiation C2C12 myotubes were treated with LG (Low glucose DMEM with 2% horse serum) for 24h and the media were changed with HG (High glucose DMEM with 2% horse serum). Total mRNA and protein were isolated. n=4. (* = $p < 0.05$ vs Veh)


100kDa
50kDa
Sortilin
γ-tubulin
2mM low glucose
24h Pretreatment
0 3 6 9 24 (h)
25mM
High glucose


Sortilin Protein
Relative Protein Levels
0 1 2 3 4 5 6
*
2mM low glucose
24h Pretreatment
0 3 6 9 24 (h)

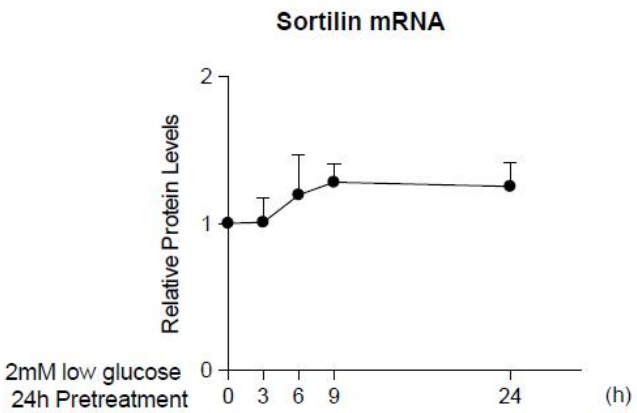

Sortilin mRNA
Relative Protein Levels
0 1 2
2mM low glucose
24h Pretreatment
0 3 6 9 24 (h)

Figure 9. High glucose condition increases sortilin protein in a time dependent manner.

C2C12 myotubes were maintained in low glucose DMEM with 2% horse serum for 24h, and then the media were replaced by high glucose DMEM for the indicated periods. n=3. (* = $p < 0.05$ vs Veh)

III. Investigation on the mechanism of sortilin posttranslational modification by glucose.

Glucose levels regulate sortilin protein stability.

Because glucose concentration regulates sortilin protein level without any change of its mRNA level, glucose may affect sortilin protein stability. To determine whether glucose indeed affects the stability of sortilin, I examined the stability of sortilin on C2C12 myotubes in the presence of cycloheximide. Sortilin under low glucose was degraded more rapidly than under the high glucose condition (Figure 10).

Because proteasome-dependent degradation of hepatic sortilin was reported, [12, 18] I tested whether glucose concentration affects proteasome-dependent degradation of sortilin in C2C12 myotubes. To examine this, C2C12 myotubes were treated low or high glucose in the presence of MG132, a proteasome inhibitor (Figure 11). In the presence of MG132, the reduction of sortilin protein in the low glucose condition was not observed anymore, suggesting that the low glucose condition facilitates proteasome-mediated degradation of sortilin

.

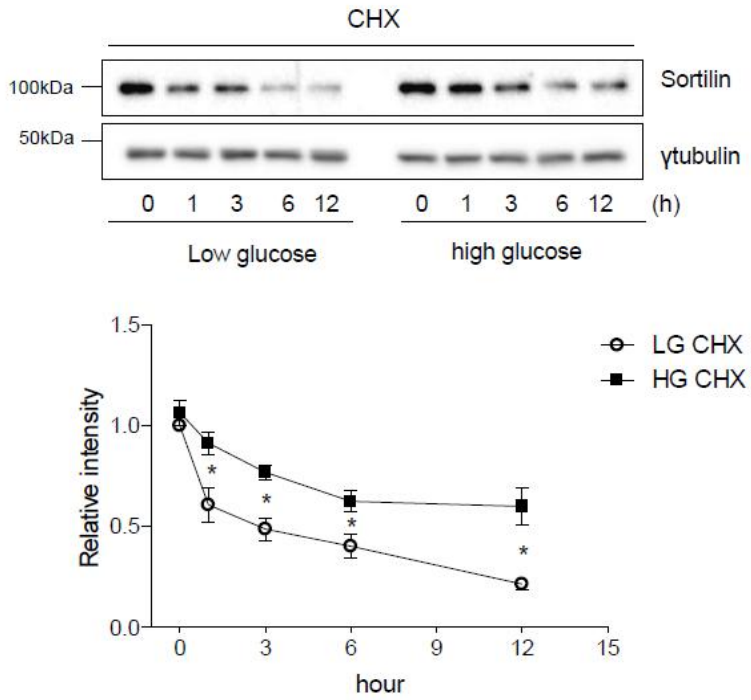


Figure 10. Effect of low glucose on sortilin stability.

C2C12 myotubes were treated high glucose or low glucose in the presence of cycloheximide (50uM) for the indicated periods. The density of the sortilin protein bands in the Western blot was measured. Mean band intensity of 4 independent blots was plotted to illustrate protein degradation rate. (* = $p < 0.05$ vs HG)

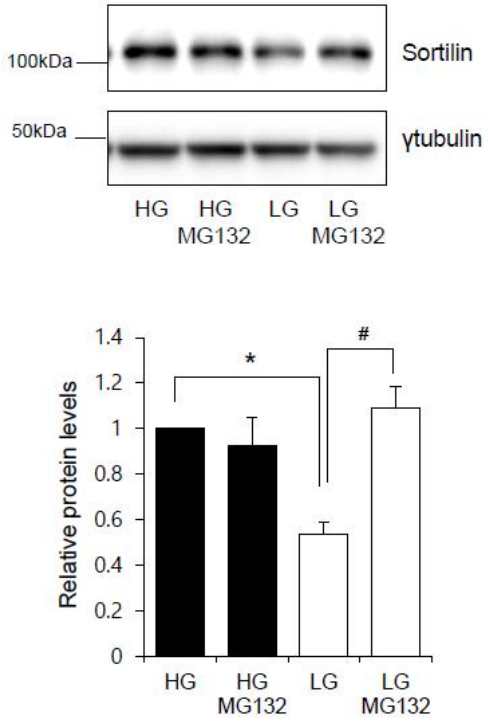


Figure 11. Effect of low glucose on the proteasome-mediated sortilin degradation.

C2C12 myotubes were treated with high glucose or low glucose in the presence of MG132 (20uM) for 4h, and then cell lysates were subjected to immunoblot analysis. n=4. (* = p <0.05 vs HG, # = p <0.05 vs LG)

Sortilin regulates insulin stimulated Glut4 trafficking.

It has been reported that insulin-stimulated glucose uptake in insulin sensitive tissues, such as muscle and adipocytes, mainly correlates with the amount of Glut4 in the plasma membrane.[14, 15, 17] In my study showed that sortilin stability was regulated by high glucose in C2C12. Therefore sortilin may play an important role in glucose uptake.

I tested whether sortilin affects glucose uptake by Glut4 translocation to plasma membrane. L6-GLUT4myc myotubes were treated with or without insulin. Sortilin knockdown decreased insulin-stimulated glucose uptake and insulin-stimulated Glut4 translocation (Figure 12 and 13). These results suggest that sortilin participates in the regulation of insulin-simulated Glut4 trafficking to plasma membrane.

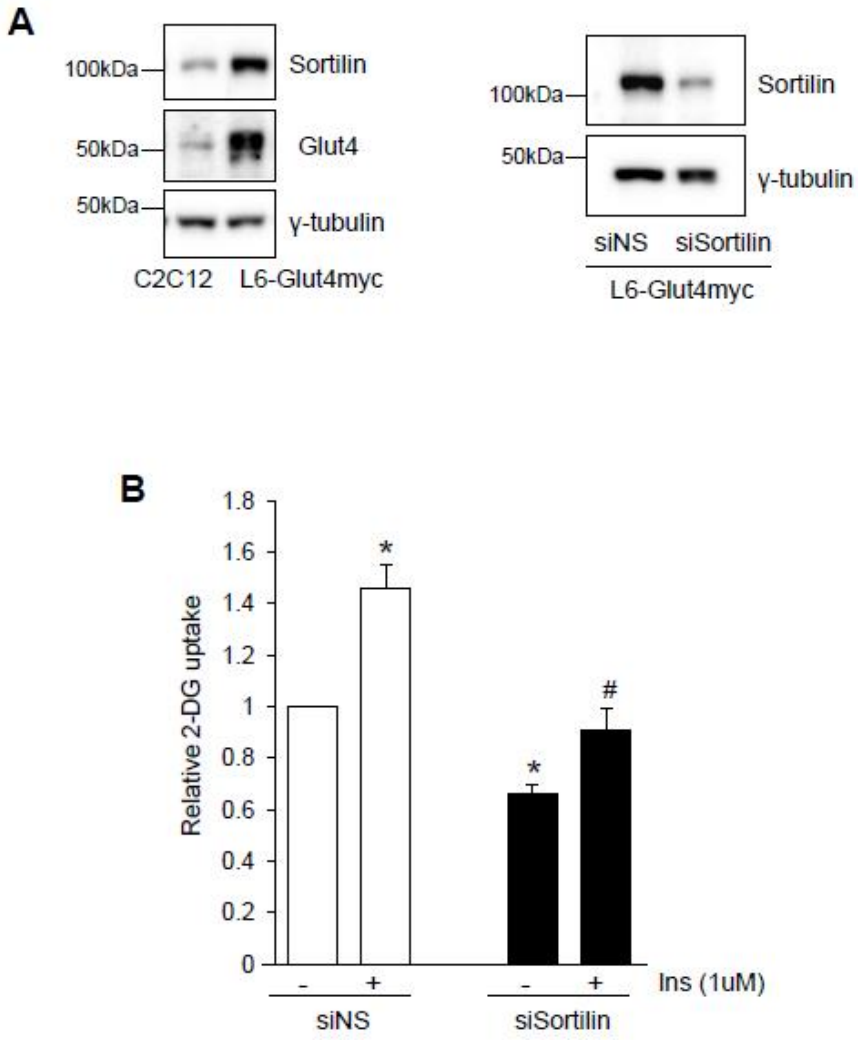
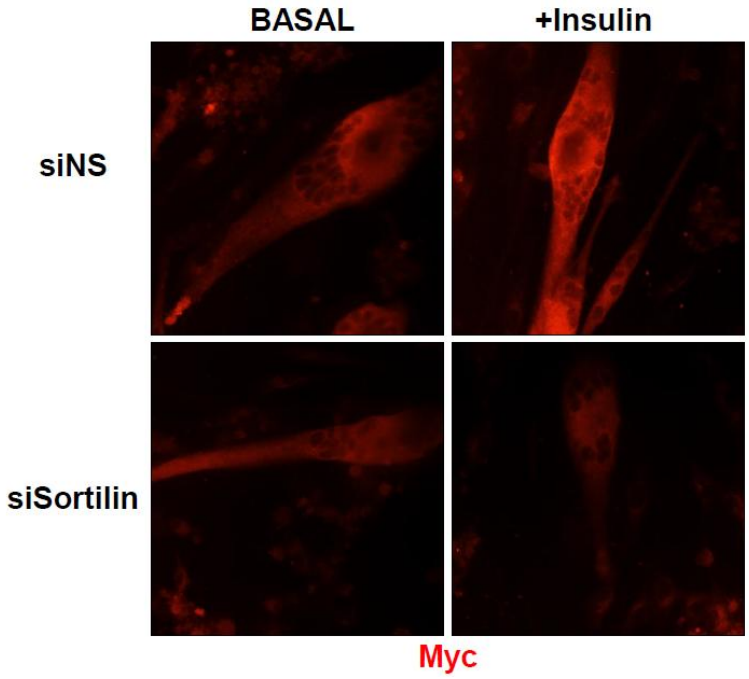

A
100kDa
Sortilin
50kDa
Glut4
50kDa
γ-tubulin
C2C12
L6-Glut4myc
100kDa
Sortilin
50kDa
γ-tubulin
siNS
siSortilin
L6-Glut4myc
B
Relative 2-DG uptake
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
#
-
+
-
+
Ins (1uM)
siNS
siSortilin

Figure 12. Sortilin knockdown decreases insulin-stimulated glucose uptake

(A) C2C12 myotubes and L6-GLUT4myc myotubes were transfected with 50nM siRNAs for 48 hours (siSortilin or siNS). (B) L6-GLUT4myc myotubes were deprived of serum 3 hours, followed by treatment with insulin (1uM) for 30 min. Glucose uptake was measured using a Glucose uptake colorimetric assay kit according to the manufacturer's protocols. n=4. (*, $p < 0.05$ vs siNS, #, $p < 0.05$ vs siNS Ins)


BASAL
+Insulin
siNS
siSortilin
Myc

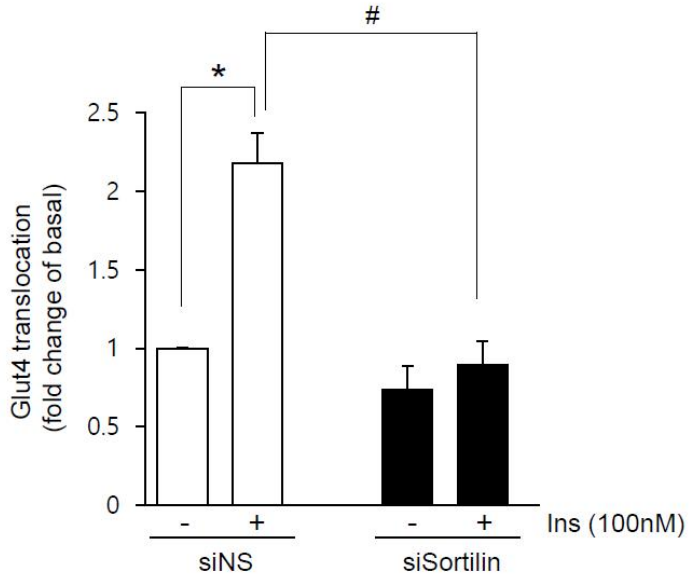

#
*
2.5
2
1.5
1
0.5
0
Glut4 translocation
(fold change of basal)
-
+
-
+
Ins (100nM)
siNS
siSortilin

Figure 13. Sortilin knockdown inhibits insulin-stimulated GLUT4myc translocation to the plasma membranes.

L6-GLUT4myc myotubes grown on glass coverslips with 0.1% gelatin were transfected with siRNAs (siNS or siSortilin). Cells were deprived of serum 3 hour before treatment with insulin (100nM) for 30 min. Myc was detected by immunofluorescence using an anti-Myc antibody. All images were captured using fluorescent microscope. n=3. (*, $p < 0.05$ vs siNS, #, $p < 0.05$ vs siNS Ins)

Discussion

There are some reports to show the role of sortilin in the liver. However, any role of sortilin in the skeletal muscle has not been revealed. In this study, I found that sortilin expression is distinct in several tissues. In the diabetic mice such as db/db and HFD-induced obese mice, sortilin protein was increased in skeletal muscle while sortilin was decreased in liver and adipose tissue. Moreover, the mRNA levels of sortiln were significantly decreased in the liver and adipose tissue from the diabetic mice. However, sortilin mRNA levels in skeletal muscle were not increased in db/db mice (Figure 1 and 2) or DIO mice (Figure 4 and 5).

Sortilin transports several proteins to cell membrane and lysosome vesicle. In addition, posttranslational modification of sortilin is important for the sortilin-mediated lysosomal degradation of ApoB100-VLDL.[12, 18] Furthermore, hepatic sortilin is highly sensitive to insulin-induced degradation and this regulation is related to sortilin ubiquitination.[19] In this study, I showed that low glucose treatment significantly decreased sortilin protein levels in myotubes, which is related

to sortilin stability regulated by proteasome-mediated degradation (Figure 8 and 10).

Here, I suggest a hypothesis to explain a functional role of sortilin, whose protein level is regulated by extracellular glucose concentration, under high glucose condition: Under high glucose condition, sortilin on the TGN becomes stable and facilitates Glut4 trafficking. Furthermore, knockdown of sortilin in L6-GLUT4myc myotubes were decreased not only insulin-stimulated glucose uptake but also basal glucose uptake (Figure 12 and 13).

On support of this hypothesis, sortilin is known to involve Glut4 translocation, which is stimulated by insulin signaling. [6, 10, 13, 20] In addition, overexpression of sortilin in C2C12 myotubes were increased not only Glut4 protein stability, but were also improved insulin stimulated glucose uptake.[6]

Here, I demonstrate that sortilin is increased in muscle cells and tissues under high glucose condition. Furthermore Glut4 translocation from intracellular storage to the plasma membrane was decreased by sortilin knockdown.

Although sortilin actions correlate with glucose metabolism, further studies will be required to implicate sortilin as a regulator of glucose transport.

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국문초록

Sortilin은 신경계에서 Vps10도메인을 가지는 nueroteinsin 수용체로 알려져 있으며, 트랜스 골지와 리소솜 사이의 리간드 수용체로써 세포 내 단백질을 수송한다. 기존 연구된 바에 따르면 sortilin은 세포 내 이동, 분비, 내포작용을 하는 것으로 알려져 있으며, 간에서 sortilin은 ApoB100의 분비를 막으며, 리소솜을 통한 LDL 분해에 관련이 있다. 하지만 골격근에서의 sortilin의 역할은 현재까지 많은 연구가 되어 있지 않다. 따라서 본 연구에서 저자는 생쥐의 골격근 세포 주 및 당뇨동물모델을 통해 sortilin의 골격근에서 발현과 역할에 대해 연구하였다.

당뇨인 생쥐의 골격근에서 sortilin의 발현을 보았을 때 그 양이 증가하는 것과는 다르게 간, 지방조직에서는 sortilin의 발현이 감소하였다. 고지방식이를 먹여 당뇨를 유발한 생쥐의 골격근에서도 역시 sortilin의 발현은 증가하나 간, 지방조직에서는 sortilin의 발현이 감소하였다. 따라서 이는 조직에 따라 sortilin의 역할과 조절이 다르다는 것을 보여준다.

따라서 당뇨, 비만과 관련 있는 인자인 포화지방산, 염증인자,

미토콘드리아 저해제, 포도당을 생쥐 골격근 세포 주인 C2C12 myotubes에 처리하여 sortilin의 발현 양을 보았다. 하지만 포화지방산인 palmitate가 간 sortilin에 중요한 영향을 미치는 것과는 달리 C2C12 myotubes에서는 sortilin의 단백질과 mRNA 발현 양이 변하지 않았다. 하지만 저농도 포도당을 C2C12 myotubes에 처리하였을 때, sortilin의 단백질양은 큰 감소를 보이나 mRNA양은 변하지 않는 것을 확인하였다.

골격근에서의 포도당에 의한 sortilin 조절을 좀 더 알아보기 위해 C2C12 myotubes에 저농도 포도당을 24시간 처리하고 다시 고농도 포도당을 처리하였다. 이 때 저농도 포도당으로 인해 감소되었던 sortilin의 단백질 양이 고농도 포도당으로 인해 증가하였다. 이 같이 mRNA가 변하지 않으나 단백질의 양이 변하는 것은 포도당이 sortilin 단백질의 안정성과 관련이 있을 거라 생각하여 실험 한 결과 저농도 포도당 조건에서 proteasome을 통해 sortilin이 분해된다는 것을 확인하였다. 이러한 결과를 통해 고농도 포도당에 의해 sortilin 단백질의 안정성이 증가함으로써 골격근에서 sortilin이 증가하는 것임을 알 수 있다. 또한 골격근에서 고농도 포도당에 의해 증가된 sortilin은 당 수송 운반체인

Glut4 운반체를 인슐린 자극에 의해 세포 내에서 세포막으로 이동시켜줄 뿐만 아니라 포도당의 흡수도 촉진하였다.

이러한 결과를 통해 sortilin은 당뇨와 같은 고농도 당 조건하에서 골격근에 발현 양이 늘어나며 이는 고농도의 당을 골격근 내로 흡수하기 위함임을 밝혀내었다. 그러므로 이는 당뇨 및 대사질환에서 sortilin을 생체표지자로써 이용할 수 있을 것이다.

주요단어 : Sortilin, 포도당, 골격근, 당 수송 운반체, 비만, 당뇨

학번 : 2015-26085