Functional Study of *O*-GlcNAcylation on ULK1 under glucose deprivation

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This certifies that the dissertation
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감사의 글

2013년, 처음 연구원의 꿈을 꾸고 처음 실험실에 들어와 3년 동안 많은 것들을 배우고 어느덧 석사로 졸업이 눈앞으로 다가왔습니다. 많이 부족한 제가 이렇게 졸업을 할 수 있도록 도와주신 조진원 교수님과 학위 과정동안 이런저런 고민들을 들어주고 응원해주신 분들에게 이렇게 글로나마 감사의 말씀들 드립니다.

먼저 저를 지도해주신 조진원 교수님께 감사드립니다. 학위 과정동안 알려주신 연구자로서의 태도와 마음가짐, 그리고 항상 강조하신 효과적인 발표 방법은 잊지 않고 항상 기억하겠습니다. 랩 미팅을 할 때 부족한 점들을 알려주셨기에 부족했던 점들을 조금이나마 채워서 졸업할 수 있었던 것 같습니다. 석사로 전환하고 나서 학위 논문이 나올 때 까지 계속 세심한 지도 해주셔서 감사하고 여기서 배운 것들을 통해 더 나은 연구자가 되도록 노력하겠습니다. 또한 저의 석사 논문을 심사해주신 구남온, 이태호 교수님께도 감사드립니다.

하루 중 저와 가장 오랜 시간을 같이 지낸 당방 연구실 멤버들에게도 감사드립니다. 먼저 요즘 준영이 때문에 고생 많으신 양신누나!, 진짜 제가 이런저런 고민이 있을 때마다 진심어린 조언해주시고 용기 주셔서 감사합니다. 그리고 언제나 저에게 실험에 대한 이야기를 아끼지 않으셨던 한별이형, 보고서 쓸 때 마다 늘 죄송했던 현규형, 저에게 항상 용기와 웃음을 주셨던 은아누나, 처음 실험실 왔을 때 이런저런 조언을 해주신 연정누나, 그리고 이렇게 갑자기 석사 전환해서 미안한 정화까지 모두모두 감사하고 여러분이 없었으면 석사 졸업이라는 결실을 못 봤을 것 같아요. 그리고 지금은 졸업해서 외국에 계신 두 분의 사수이자 조언자이신 박수진, 장인숙 박사님!, 정말 두 분이 저에게 하나부터 열까지 알려주시고 이끌어주신 덕에 부족한 제가 이렇게 졸업 할

수 있게 되었네요. 정말 고맙고 항상 모든 일 잘 되기를 기도할께요. 또 저에게 석사 졸업 준비에 많은 조언을 해주고 고민 상담을 해준 주환이형이랑 지영이에게도 고맙다는 말 전하고 싶어요.

학위과정 동안에 힘든 일 있을 때 짜증 받아주고 응원과 격려해준 우리가족, 고마워요! 주말에 실험한다고 어디 갈 때 같이 못가서 미안하고 특히 진현이 주말에 같이 못 놀아줘서 미안해.

학부 때부터 계속 인연이 되는 구조방에서 고생하고 있을 준배야, 웨스턴 어렵지 않아ㅎㅎㅎㅎ, 그리고 옆에 있을 정석아 먼저 졸업해서 미안, 과학원 지하 일층에서 또 고생할 군대 알동기 태윤아 너 덕에 칩 쫌 잘 해결되서 고마워. 그리고 처음 성인이 되고 만난 우리 매지3학사 RA분반 친구&형들 힘을 줘서 고마워!

마지막으로 힘들 때마다 욕 많이 했을 텐데 그거 들어주시느라 고생 많았던 하느님에게도 고맙다는 말 전하고 싶습니다.

박사가 아닌 석사로 졸업하게 돼서 아쉬움이 조금은 남는 것은 사실이지만 새로운 시작을 앞두고 있어서인지 설렘도 크네요. 앞으로도 열심히 공부해서 좋은 연구자가 되도록 노력하겠습니다, 학위 기간 동안 도움 주신 많은 분들께 다시한번 감사의 말씀을 드리면서 이 글을 마치겠습니다.

2016.6.22.

박진원 올림

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Chapter 3. Results

Abbreviation

AMPK : AMPK activated protein kinase

ATG : autophagy related protein

CTL : control

HBP : hexosamine biosynthetic pathway

LC3 : microtubule associated protein 1 light chain 3 beta

mTOR : mammalian target of rapamycin

O-GlcNAc : *O*-linked β-*N*-acetylglucosamine

OGT : *O*-GlcNAc transferase

OGA : *O*-GlcNAcase

sWGA : succinylated wheat germ agglutinin

UDP-GlcNAc : uridine 5'-diphospho-*N*-actylglucosamine

ULK1 : unc-51 like autophagy activating kinase 1

WT : wild type

Abstract

O-GlcNAcylation is a dynamic posttranslational modification that occurs in the serine or threonine residues of nuclear and cytosolic proteins. *O*-GlcNAcylation regulates various cellular events, such as a nutrient sensing, epigenetic regulation, translational regulation, and cell proliferation, as well as disease-related signal pathways. UDP-GlcNAc, a precursor of *O*-GlcNAcylation, is synthesized from glucose by the hexosamine biosynthetic pathway (HBP). The level of UDP-GlcNAc depends on the cellular glucose condition. For this reason, *O*-GlcNAcylation is called a nutrient sensor. Although *O*-GlcNAcylation plays a role in nutrient sensing and metabolism, the relationship between *O*-GlcNAcylation and autophagy remains unknown. In previous studies, decreased *O*-GlcNAcylation by OGT siRNA induced autophagy without starvation. In this study, we observed that a unc-51 like autophagy activating kinase 1(ULK1), a regulator of autophagy, is *O*-GlcNAcylated. Under glucose deprivation, *O*-GlcNAcylated ULk1 is increased, and increased *O*-GlcNAcylation induces ULK1 S757 phosphorylation, which leads to ULK1 inactivation. The ULK1 S757A mutant decreases *O*-GlcNAcylation on ULK1 and binding affinity with OGT compared with ULK1 wild type. These data imply that *O*-GlcNAcylation on ULK1 decreases ULK1 kinase activity by increasing S757 phosphorylation and reduces autophagy induction.

Chapter 1. Introduction

O-GlcNAcylation is a dynamic posttranslational modification that occurs in the serine or threonine residues, the same residues involved in phosphorylation, of nuclear and cytosolic proteins [1]. The *O*-GlcNAc cycle is dynamically regulated by *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA). UDP-GlcNAc, a substrate for *O*-GlcNAcylation, is synthesized through the hexosamine biosynthetic pathway (HBP) [2, 3]. Therefore, UDP-GlcNAc is influenced by glucose, fatty acid, uridine, and glutamine, which is why *O*-GlcNAcylation is called a nutrient sensor indicating nutrient conditions [4-6].

Autophagy plays a role in the degradation of cellular components and organelles under starvation, cellular stress or dysfunction, and development [7]. In previous studies about the relationship between *O*-GlcNAcylation and autophagy, researchers found that decreased *O*-GlcNAcylation by the loss of OGT induced autophagy without starvation. They also confirmed *O*-GlcNAcylation on the ATG gene transcriptional factor dFOXO and SNARE complex component SNAP-29, which interrupted ATG gene transcription and autophagy degradation [8-10]. dFOXO and SNAP-29 regulate ATG gene transcription and autophagy degradation but not autophagy formation.

Thus, we focused on the unc-51 like autophagy activating kinase 1

(ULK1). ULK1 is the sole kinase of the identified ATG family [11]. ULK1 initiates autophagy formation, and it is regulated by AMPK and mTOR. AMPK induces ULK1 kinase activity during the induction of autophagy and phosphorylates the ULK1 S555 residue. mTOR reduces ULK1 kinase activity during the reduction of autophagy and phosphorylates the S757 residue [12, 13].

In this study, we demonstrate that ULK1 is *O*-GlcNAcylated. Under the conditions of increased ULK1 *O*-GlcNAcylation, ULK1 S757 phosphorylation, which reduces ULK1 kinase activity, was found to increase. Moreover, ULK1 *O*-GlcNAcylation and OGT binding affinity decreased in the ULK1 S757A mutant. Such results demonstrate the functional effect of *O*-GlcNAcylation on ULK1.

Chapter 2. Materials and Methods

2.1 Cell culture and glucose deprivation

Hek293 (human embryonic kidney cell) cell lines were cultured in high-glucose (25mM) DMEM (Dulbecco's Modified Eagle's Medium, Lonza) supplemented with 10% FBS (Fetal Bovine Serum, Biotechnics Research) and 1% P/S (Penicillin/Streptomycin, Gibco) in 5% CO_2 at 37℃. Cells were rinsed with PBS and then cultured in glucose-free DMEM (Sigma-Aldrich) supplemented with 10% FBS (Biotechnics Research) and 1% P/S (Gibco) for the indicated amount of time [14].

2.2 DNA plasmids and transfection

Human full-length HA-ULK1 WT, HA-ULK1 S757A, and HA-ULK1 S757D were kindly provided by Prof. Joungmok Kim (Kyunghee University, Seoul, Korea) [15]. Untagged pCMV-OGT was used in OGT overexpression. These DNA plasmids were transfected using polyethylenimine (PEI, Sigma Aldrich).

2.3 Cell lysis and Western blot

Cells were lysed using RIPA buffer [50mM Tris-HCL, pH 7.4, 150mM NaCl, 1% Nonidet p-40, 1% SDS, 2mM EDTA, 0.5% deoxycholic acid, 1mM NaF, and protease inhibitor cocktail (Roche Applied Science)] on ice for 30 min with vortexing every 5 min and centrifuged at 14,000rpm

for 20min. Protein concentrations of the lysates were measured by Bradford Assay using Protein Assay Dye Reagent Concentrate (BIO-RAD). The lysates containing 20μg of protein were boiled in 4x SDS sample buffer for 5 min. The samples were separated by SDS-PAGE using 10-14% polyacrylamide gel and transferred to nitrocellulose membranes (GE Healthcare). The membranes were incubated in TBS-T containing 5% skimmed milk for 1 hour at room temperature and washed three times with TBS-T. After washing, the membranes were incubated in primary antibodies diluted in TBS-T overnight at 4℃ and washed three times with TBS-T. They were incubated in secondary antibodies diluted in TBS-T for 1 hour at room temperature and washed three times with TBS-T. Immunoreactive bands were detected using an ECL system (GE Healthcare, Dogen). Quantification was performed using Multi Gauge V3.1 (Fujifilm).

2.4 Succinylated wheat germ agglutinin (sWGA) precipitation and immunoprecipitation

For sWGA precipitation, 2mg of cell lysates were incubated with lysis buffer-washed agarose-sWGA (Vector Lab) overnight at 4℃. For immunoprecipitation, 2mg of cell lysates were incubated with lysis buffer-washed protein A/G agarose beads (Santa Cruz) coated with rabbit HA (Sigma-Aldrich) overnight at 4℃. After washing five times with lysis buffer, precipitates were eluted by 2x SDS sample buffer and boiled for 5 min.

2.5 Antibodies

The following primary antibodies were used: *O*-GlcNAc (anti-RL2 (Thermo Scientific, MA1-072), anti-CTD 110.6 (Convance, MMS-248R)), anti-OGT (Santa Cruz, H300), anti-β-actin (Cell Signaling, 4967), anti-LC3 (MBL, PM036), anti-ULK1 (Santa Cruz, sc-33182), anti-phospho S555-ULK1 (Genetex, GTX20207), anti-phospho S757-ULK1 (Cell Signaling, 68885), and anti-HA (Sigma-Aldrich, H3663).

The secondary antibodies conjugated to HRP (Horse radish peroxidase) were purchased from Santa Cruz Biotechnology (anti-mouse IgG; sc-2005, anti-mouse IgM; sc-2064, anti-rabbit IgG; sc-2004).

2.6 Statistical analysis

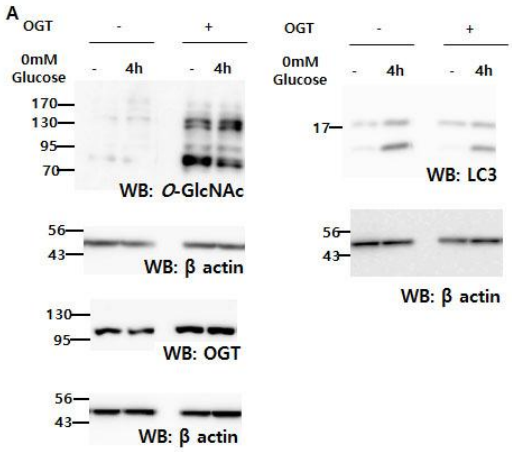
Statistical significance was determined by a two-tailed student's t test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). All results are given as the mean ±standard error based on at least three independent experiments.

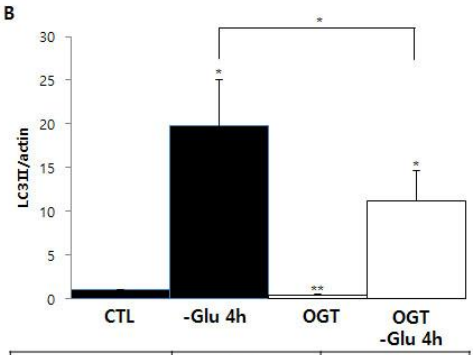
Chapter 3. Results

3.1 Increased global *O*-GlcNAcylation level by OGT overexpression decreases autophagy induction

In a previous study, increased *O*-GlcNAcylation reduced autophagy flux only in *drosophila melanogaster* [9]. Thus, we confirmed human cell lines Hek 293. We increased *O*-GlcNAcylation by OGT overexpression and treated glucose deprivation for 4 hours to induce autophagy. We assayed the level of LC3 II, an autophagic marker, by Western blot [16, 17]. The level of LC3 II was decreased in OGT-overexpressed cells (Fig. 1A). Without glucose deprivation, OGT overexpression reduced autophagy induction by 59% compared with the vector. Under glucose deprivation, OGT overexpression reduced autophagy induction by 43% (Fig. 1B).

Through these results, we showed that increased *O*-GlcNAcylation by OGT overexpression decreased autophagy induction.





Glucose deprivation	Vector		OGT	
	-	4h	-	4h
LC3Ⅱ	**1**	**19.73**	**0.41**	**11.23**
Standard error	0	5.36	0.09	3.39
P value		0.0251	0.0023	0.0391
		-	-	0.0331

Figure 1. Increased global *O*-GlcNAc level by OGT overexpression decreases autophagy induction. (A) Untagged OGT was overexpressed in cells. After glucose deprivation treatment, harvested cells with indicated time points were analyzed by Western blot. LC3, *O*-GlcNAc, and β-actin were immunoblotted using individual antibodies. (B) Quantified LC3 II values are displayed in graph.

3.2 ULK1 is *O*-GlcNAcylated under OGT overexpression

We found that *O*-GlcNAcylation interrupts autophagy formation by previous experiments, and we confirmed *O*-GlcNAcylation on some ATG proteins that regulate autophagy formation. We examined whether ULk1 is *O*-GlcNAcylated. When HA-ULK1 and OGT were overexpressed, ULK1 was detected by the *O*-GlcNAc antibody (Fig. 2).

In this experiment, we demonstrated *O*-GlcNAcylation on ULK1.

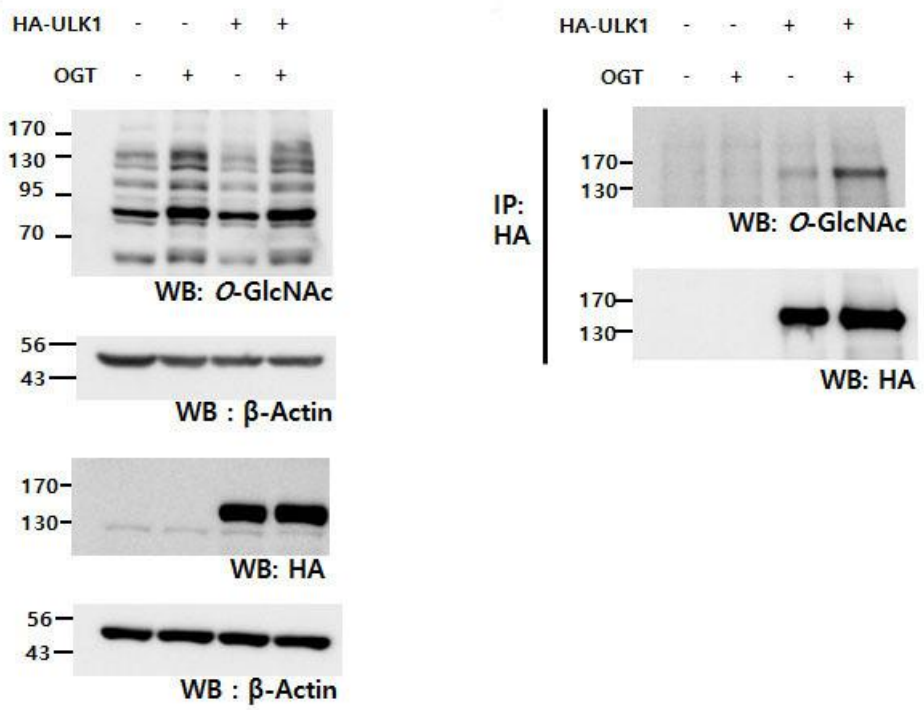


Figure 2. ULK1 protein is *O*-GlcNAcylated. HA-tagged ULK1 (HA-ULK1) was overexpressed together with untagged OGT in cells. After immunoprecipitation with HA affinity gel, *O*-GlcNAcylated HA-ULK1 was detected. Overexpressed ULK1 was detected by HA antibody. *O*-GlcNAc, OGT, and β-actin were immunoblotted using individual antibodies.

3.3 Increased *O*-GlcNAcylation increases ULK1 S757 phosphorylation

To confirm whether *O*-GlcNAcylation on ULK1 is regulated under autophagy induction, we performed glucose deprivation treatment for 0.5 to 4 hours. The harvested cells were then used in lectin precipitation with sWGA that binds the *O*-GlcNAcylated proteins. We confirmed that ULK1 S757 phosphorylation, which reduces ULK1 kinase activity, was increased from 0.5 to 2 hours, and ULk1 S555 phosphorylation, which induces ULK1 kinase activity, was also increased from 0.5 to 2 hours. At that time, *O*-GlcNAcylation on ULK1 was increased from 0.5 to 2 hours and peaked at 2 hours (Fig. 3).

To observe how a change in *O*-GlcNAcylation on ULK1 regulated ULK1 phosphorylation, we overexpressed OGT to increase *O*-GlcNAcylation on ULK1 and performed glucose deprivation treatment for 2 or 4 hours. The level of LC3 II was found to increase time dependently with glucose deprivation. At 0 and 2 hours, ULK1 S757 phosphorylation was increased by increased *O*-GlcNAcylation but not ULK1 S555 phosphorylation (Fig. 4A). Increased *O*-GlcNAcylation induced 20% of ULK1 S555 phosphorylation (Fig. 4B).

Taken together, we concluded that *O*-GlcNAcylation on ULK1 was essential for autophagy induction and reduced ULK1 kinase activity by increased ULK1 S757 phosphorylation.

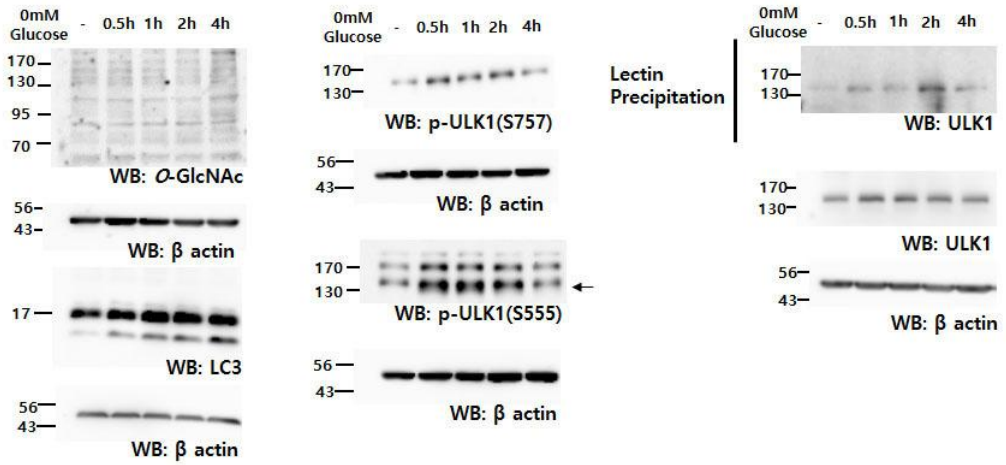
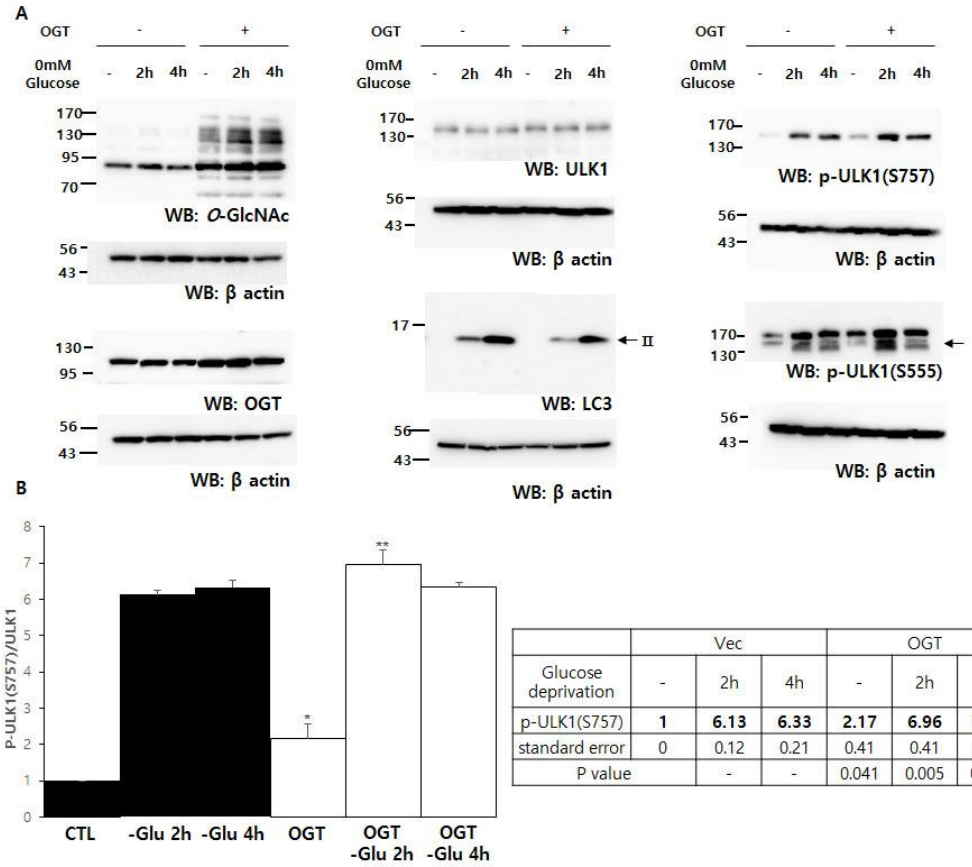


Figure 3. *O*-GlcNAcylated ULK1 is essential for autophagy induction. After glucose deprivation treatment, harvested cells with indicated time points were analyzed by Western blot. Endogenous ULK1 was precipitated using sWGA. LC3, *O*-GlcNAc, ULK1, ULK1 S555 phosphorylation, ULK1 S757 phosphorylation, phospho S757 ULK1, and β-actin were immunoblotted using individual antibodies.



	Vec			OGT		
Glucose deprivation	-	2h	4h	-	2h	4h
p-ULK1(S757)	**1**	**6.13**	**6.33**	**2.17**	**6.96**	**6.35**
standard error	0	0.12	0.21	0.41	0.41	0.11
P value		-	-	0.041	0.005	0.928

Figure 4. Increased *O*-GlcNAcylation increases ULK1 S757 phosphorylation. (A) Untagged OGT was overexpressed in cells. After glucose deprivation treatment, harvested cells with indicated time points were analyzed by Western blot. LC3, *O*-GlcNAc, OGT, ULK1, ULK1 S555 phosphorylation, ULK1 S757 phosphorylation, and β-actin were immunoblotted using individual antibodies. (B) Quantified ULK1 S757 phosphorylation values are displayed in graph.

3.4 ULK1 S757 phosphorylation has negative effect on OGT binding and *O*-GlcNAcylation on ULK1

A previous experiment determined that the level of ULK1 S757 phosphorylation was regulated by *O*-GlcNAcylation on ULK1. and we aimed to determine the relationship between *O*-GlcNAcylation on ULK1 and ULK1 S757 phosphorylation. We overexpressed ULk1 or the ULK1 S757A mutant that suppresses ULK1 S757A together with OGT. *O*-GlcNAcylation on the ULK1 S757A mutant was decreased compared with *O*-GlcNAcylation on ULK1, because the ULk1 S757 mutant reduced the binding affinity of OGT (Fig. 5). Moreover, we overexpressed ULK1 of the ULK1 S757A mutant and the ULK1 S757D mutant, which is a phosphorylation mimic. *O*-GlcNAcylation on ULK1 S757D did not change, unlike *O*-GlcNAcylation on ULK1 (Fig. 6).

Taken together, we concluded that ULK1 S757 phosphorylation has a negative effect on the *O*-GlcNAcylation of ULK1 and OGT binding.

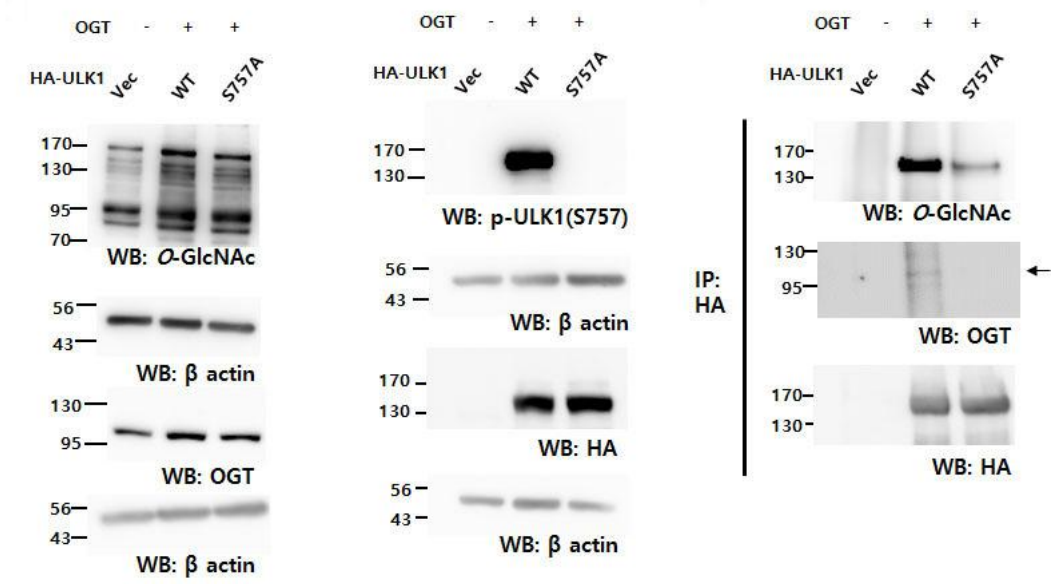


Figure 5. S757 phosphorylation has negative effect on OGT binding and *O*-GlcNAcylation on ULK1. HA-ULK1 or HA-ULK1 S757A mutant were overexpressed together with untagged OGT in cells. After immunoprecipitation with HA affinity gel, *O*-GlcNAcylated HA-ULK1 and HA-ULK1 S757A mutant were detected. Overexpressed ULK1 and ULK1 S757A mutant were detected by HA antibody. *O*-GlcNAc, OGT, ULK1 S757 phosphorylation, and β-actin were immunoblotted using individual antibodies.

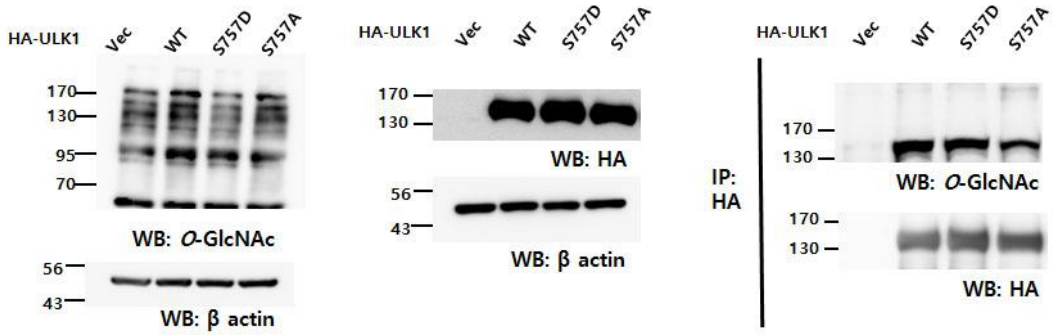


Figure 6. S757 phosphorylation mimic (S757D) has no effect on *O*-GlcNAcylation on ULK1. HA-ULK1 or HA-ULK1 S757A or HA-ULK1 S757D mutant were overexpressed together with untagged OGT in cells. After immunoprecipitation with HA affinity gel, *O*-GlcNAcylated HA-ULK1, HA-ULK1 S757A mutant, and HA-ULK1 S757D mutant were detected. Overexpressed ULK1, ULK1 S757A mutant, and ULK1 S757D mutant were detected by HA antibody. *O*-GlcNAc, and β-actin were immunoblotted using individual antibodies.

Chapter 4. Discussion

In previous studies, the relationship between *O*-GlcNAcylation, the nutrient sensor in cells, and autophagy was reported. Decreased *O*-GlcNAcylation induced autophagy formation and degradation. However, the effect of autophagy formation under changing *O*-GlcNAcylation has not yet been studied in detail.

In this study, we demonstrated that *O*-GlcNAcylation on ULK1 decreases ULk1 kinase activity. This study showed that *O*-GlcNAcylation directly regulates autophagy formation. However, there were several limitations in this study. First, although the ULK1 S757A mutant reduced *O*-GlcNAcylation on ULK1 compared with ULK1, we did not define a specific *O*-GlcNAcylation site on ULK1, and we could not confirm the exact function of *O*-GlcNAcylation on ULK1. In further studies, there is a need to identify the *O*-GlcNAcylation sites of ULK1 by using mass spectrometry and point mutation. By obtaining results using the ULK1 *O*-GlcNAcylation site mutant, it will be possible to prove that *O*-GlcNAcylation on ULK1 directly regulates S757 phosphorylation.

Second, in Figure 6, the ULK1 S757D mutant, a phosphorylation mimic, did not induce *O*-GlcNAcylation on ULK1, unlike ULK1. The ULK1 S757 phosphorylation mimic probably maintains high levels of phosphorylation under fed conditions (Fig. 5). From this experiment

alone, we cannot conclude that ULK1 S757 phosphorylation is essential for OGT binding with ULK1 and *O*-GlcNAcylation on ULK1.

Lastly, we confirmed that the overexpression of the ULK1 S757A mutant reduced the binding affinity of OGT. However, we cannot explain this phenomenon through experiments. Because mTOR, which phosphorylates ULK1 S757, leads to conformational changes of ULK1, we hypothesize that the ULK1 conformationally changed by increased ULK1 S757 phosphorylation reduces the binding affinity of OGT [18].

Regardless of these limitations, this research provides a novel understanding of the relationship between *O*-GlcNAcylation and autophagy formation-mediated *O*-GlcNAcylation on ULK1. Thus, we can demonstrate the function of *O*-GlcNAcylation on ULK1 under autophagy induction by glucose deprivation.

Summary

1. Increased global *O*-GlcNAc level by OGT overexpression decreases autophagy induction.

2. ULK1 is *O*-GlcNAc modified upon OGT overexpression.

3. *O*-GlcNAcylated ULK1 is essential for autophagy induction.

4. Increased *O*-GlcNAcylation on ULK1 increases ULK1 S757 phosphorylation.

5. The ULK1 S757A mutant decreases *O*-GlcNAcylation on ULK1 and OGT interaction

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국문 요약

O-GlcNAcylation은 핵과 세포질에 존재하는 단백질의 세린 혹은 트레오닌 잔기에서 일어나는 단당 번역 후 수식화로 단백질의 기능, 분해, 위치 등을 변화 시키는 것으로 알려져 있다. 인산화와 같은 세린, 트레오닌 잔기에서 수식화가 일어나기 때문에 *O*-GlcNAcylation은 인산화와 다양한 상호작용하여 단백질의 기능 변화 등을 유도하게 된다. *O*-GlcNAcylation는 UDP-GlcNAc을 단백질에 수식화 시키는 OGT와 다시 떨어트리는 OGA 두 효소에 의해 조절된다. *O*-GlcNAcylation의 재료로 사용되는 UDP-GlcNAc은 세포로 들어온 포도당의 2-5%가 HBP를 통해 생성되는데 이러한 이유로 *O*-GlcNAcylation은 세포 내 포도당의 양에 민감하게 반응하며 세포 내 영양상태 센서로 불리기도 한다.

또 다른 세포 내 영양상태 센서인 자가소화작용은 영양결핍상태뿐만 아니라 오래되거나 기능을 상실한 단백질 혹은 세포소기관을 제거, 그리고 발생과정 등에서 다양한 신호에 의해 활성화 된다. 영양상태 센서로 작동하는 *O*-GlcNAcylation과 자가소화작용의 연관관계에 관한 선행연구에서 *O*-GlcNAcylation을 감소시켰을 때 영양결핍이 없음에도 자가소화작용이 활성화 되는 현상을 보았고 ATG 단백질의 전사인자인 dFOXO와 Autolysosome 분해 과정에 관여하는 SNAP-29의 *O*-GlcNAcylation이 이러한 현상에 관여하는 것을 확인하였다. 하지만 아직 자가소화작용의 신호전달체계에 관여하는 *O*-GlcNAcylation되는 단백질이나 그 기능은 보고되지 않았다.

따라서 본 논문에서는 자가소화작용 신호전달의 시작점이자 유일한 인산화 효소인 ULK1의 *O*-GlcNAcylation을 밝히고 그 기능 확인에 중점을 두었다. 자가소화작용을 촉진시킨 후 ULK1의 *O*-GlcNAcylation을 sWGA 렉틴 침

강법을 통해 어떤 패턴으로 변화하는 확인하였고, *O*-GlcNAcylation을 증가시켰을 때 ULK1의 활성도를 확인 할 수 있는 인산화의 변화를 확인하였다. 그리고 *O*-GlcNAcylation에 의해 변화하는 ULK1의 인산화 자리와 *O*-GlcNAcylation간의 연관관계를 밝히기 위해 해당 인산화를 억제시키거나 촉진 시킨 돌연변이를 이용해 ULk1의 *O*-GlcNAcylation에 어떤 영향을 주는지 알아보았다.

이러한 실험을 통해 ULK1의 *O*-GlcNAcylation이 ULK1의 S757 인산화를 촉진시켜 ULK1의 활성을 감소시키는 것을 보았고 S757 인산화가 ULK1과 OGT의 결합에 관여하는 것을 보았다, 이를 통해 *O*-GlcNAcylation가 ULK1을 통해 자가소화작용에 직접적으로 관여하는 것을 확인하였다.

Keywords : *O*-GlcNAc, OGT, ULK1, Autophagy, mTOR,