Master's Thesis

석사 학위논문

Valosin-Containing Protein (VCP)/p97 is a key mediator between autophagic cell death and apoptosis in adult hippocampal neural stem cells following insulin withdrawal

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by

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A thesis submitted to the faculty of DGIST in partial fulfillment of the requirements for the degree of Master of Science in the Department of Brain and Cognitive Sciences. The study was conducted in accordance with Code of Research Ethics[1].

06. 01. 2015
Approved by

Professor Seong-Woon Yu (Signature)
(Advisor)
Professor Jae-Eun Jang (Signature)
(Co-Advisor)

1) Declaration of Ethical Conduct in Research: I, as a graduate student of DGIST, hereby declare that I have not committed any acts that may damage the credibility of my research. These include, but are not limited to: falsification, thesis written by someone else, distortion of research findings or plagiarism. I affirm that my thesis contains honest conclusions based on my own careful research under the guidance of my thesis advisor.

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Master of Science

06. 01. 2015

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Abstract

Programmed cell death (PCD) plays essential roles in regulation of survival and function of neural stem cells (NSCs). Abnormal regulation of this process is associated with aging and neurodegenerative diseases. However, the mechanisms underlying the PCD of NSCs remain largely unknown. Therefore, understanding the mechanism of PCD in NSCs is crucial for exploring therapeutic strategy for the treatment of neurodegenerative diseases.

We have previously reported that adult rat hippocampal neural stem (HCN) cells undergo autophagic cell death (ACD) following insulin withdrawal without apoptotic signs despite their normal apoptotic capabilities. It is unknown how interconnection between ACD and apoptosis is mediated in insulin-deprived HCN cells. Valosin-containing protein (VCP)/p97 is known to be essential for autophagosome maturation in mammalian cells. In this study, we report that VCP regulates the rate of autophagic flux in HCN cells following insulin withdrawal, suggesting the novel roles of VCP at other steps of autophagy as well as maturation. Particularly, VCP is expressed abundantly in HCN cells compared to hippocampal tissue and neurons. Pharmacological and genetic inhibition of VCP significantly decreased ACD and autophagy markers, while apoptotic cell death was induced in insulin-depleted HCN cells.

Taken together, these data demonstrate that VCP may play an essential role in completion of ACD and mediation of crosstalk between ACD and apoptosis in HCN cells following insulin withdrawal. Elucidating the mechanism by which VCP regulates the crosstalk of ACD and apoptosis will contribute to understanding the molecular mechanism of PCD in NSCs.

Keywords: Autophagic cell death, Apoptosis, Adult neural stem cells, Insulin withdrawal, Valosin-Containing Protein/p97

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1. Introduction

Programmed cell death (PCD) is a process by which cells deliberately destroy themselves. Abnormal regulation of this process is associated with a wide variety of human diseases, including immunological and developmental disorders, neurodegenerative diseases and cancer (1). PCD is characterized by morphological criteria (2). Type I cell death, known as apoptosis, is characterized by cell shrinkage, nuclear condensation, membrane blebbing, mitochondria dysfunction, lipid arrangement changes of plasma membrane, and nuclear DNA fragmentation. Lastly, cells are rapidly eliminated by phagocytosis (3). Type II PCD refers to autophagic cell death (ACD). Autophagy is a catabolic process that disposes of various cytoplasmic components, including protein aggregates and damaged organelles. The components are sequestered by autophagosomes, which fuse with lysosomes for degradation. This process usually occurs in response to stress to protect the cells. However, prolonged autophagy causes ACD which is named as ACD (4). Type III cell death, called necrosis, can be best defined through its morphology. It's characterized by cell swelling, dysfunction of mitochondria, rupture of cellular membrane and spill of cytoplasmic content to the extracellular space and cell lysis. Inflammatory reactions are frequently triggered in response to necrosis (5).

Autophagy is the major cellular pathway for the degradation of long-lived proteins and damaged organelles. It involves the rearrangement of subcellular membranes to sequester cargo for delivery to the lysosome where the sequestered components are degraded and recycled. For many decades, it has been known that autophagy occurs in a wide range of eukaryotic organisms and in multiple different cell types during starvation, cellular and tissue remodeling, and cell death (6-7). Based on many studies, autophagy is a protective process by eliminating damaged proteins and organelles in normal state, but excessive autophagy can cause cell death. Despite the emerging role of autophagy in regulation of PCD, the underlying mechanisms are poorly understood.

Previous study by Yu et al., (2008) reported hippocampal neural stem (HCN) cells undergo ACD upon insulin withdrawal. Cell death induced by insulin depletion did not show apoptotic sign. Instead autophagic markers were significantly increased, whereas anti-apoptotic proteins, Bcl-2 and Bcl-X_L, were decreased upon insulin withdrawal. Importantly, cell death rate was significantly decreased with

knockdown of Atg7 in insulin-deprived HCN cells (8). Currently, HCN cell death induced by insulin depletion is regard as a genuine model of ACD.

Valosin-containing protein (VCP) is a ubiquitously expressed protein belonging to the AAA+ (ATPases Associated with diverse cellular Activities) protein family with two ATPase domains, D1 and D2. Following binding of the substrates to the N and C terminal domains VCP hydrolyses ATP on its ATPase domains. VCP complex formation is changed to get its multicellular functions (9). Previous studies have reported that VCP is involved in multiple cellular processes, including cell cycle regulation, nuclear envelope formation, Golgi biogenesis, ubiquitin proteasome system and the autophagosome maturation (10). Ju et al. (2009) demonstrated that cells with loss of VCP activity failed to undergo autophagosome and lysosome fusion, thereby preventing autophagosome maturation (11-12).

Valosin-containing protein is a positive regulator of autophagosome maturation in mammalian cells. Inhibition of VCP failed autophagosome maturation and caused many neurodegenerative diseases. These previous studies prompted us to propose the involvement of VCP in regulation of ACD in HCN cells following insulin withdrawal. In this report we found the novel function of VCP in HCN cells. Pharmacological and genetic inhibition of VPC decreased autophagy initiation and induced apoptosis suggesting that VCP may play a role in autophagy initiation and mediation in crosstalk of ACD and apoptosis in insulin-deprived HCN cells following insulin withdrawal.

2. Materials and methods

2.1 Antibodies and reagents

The antibodies and reagents were used: VCP (Cell Signaling Technology, Danvers, MA, http://www.cellsignal.com; #2649), LC3B (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com; L7543), SQSTM1/p62 (Cell Signaling Technology; #5114S), β-actin (Cell Signaling Technology; #4967S), ubiquitin (Dako, Glostrup, Denmark, http://www.dako.com; Z0458), and cleaved caspase-3 antibodies (Cell Signaling Technology; #9661). *N*^{2}, *N*^{4}-dibenzylquinazoline-2,4-diamine (DBeQ) (Sigma-Aldrich; SML0031) was diluted in dimethyl sulfoxide at appropriate concentrations. Bafilomycin A1 (BafA1, Sigma-Aldrich; B1793), Z-VAD-FMK (R&D Systems, Minneapolis, MN, http://www.rndsystems.com; FMK001), necrostatin-1 (Enzo Life Sciences; BML-AP309) and lactacystin (Sigma-Aldrich; L6785) were diluted in dimethyl sulfoxide and pure water at appropriate concentrations.

2.2 Cell culture

HCN cells were grown as described previously by Chung et al (2015).

2.3 Plasmids, small interfering RNAs (siRNA)s, and transfection

HCN cells were transfected with siRNAs specific for rat VCP (Dharmacon, Lafayette, CO, http://www.dharmacon.gelifesciences.com; L-095383-02-0005) by using a Nucleofector Kit (Lonza, Basel, Switzerland, http://www.lonza.com; V4XP-4024) according to the manufacturer's instructions with minor modification. Typically, 1.5×10^5 cells/ml were suspended in an appropriate volume of Nucleofector Kit solution per reaction and transfection was performed using a 4D-Nucleofector (Lonza). After the nucleofection, the cells were seeded in plates according to the experimental

designs.

2.4 Cell death assay

HCN cells were seeded in a 96-well plate at a cell density of 1×10^5 cells /ml. Hoechst 33342 (Invitrogen, Carlsbad, CA, http://www.invitrogen.com; H3570) and propidium iodide (PI; Sigma-Aldrich; P4170) was diluted in PBS. Diluted Hoechst and PI was incubated in HCN cells (1% volume of media in the well, final 1/1000 dilution) for 20 minutes at 37°C. Cells were counted under a fluorescence microscope (Carl Zeiss, Oberkochen, Germany, http://www.zeiss.com; Axiovert 40 CFL) and collected images were analyzed using Pixcavator IA.

2.5 Western blotting

HCN cells were harvested and lysed in radioimmunoprecipitation assay buffer (Sigma-Aldrich; R0278) containing 1× protease cocktail inhibitors (Thermo Scientific; 87786) and 1× phosphatase cocktail inhibitors (Thermo Scientific; 78420) for 30 min on ice. Following centrifugation (12,000 *g*, 10 min), protein concentrations of the lysates were measured using the BCA protein assay reagent (Thermo Scientific; 23224). Typically, 10-20 µg protein per well was loaded into the gel and electrotransferred to polyvinylidene fluoride membrane (PVFM) with a semi-dry electrophoretic transfer cell (Bio-Rad, Richmond, CA, http://www.bio-rad.com). Membranes were blocked for 1 h at room temperature in 5% nonfat dry milk blocking solution, 0.1% Tween 20, and phosphate-buffered saline (PBS), pH 7.4. The membranes were then incubated overnight with the primary antibodies diluted according to the manufacturers' recommendations. The membranes were washed three times for 10 min. Membranes were incubated for 1 hour at room temperature with peroxidase-conjugated secondary antibodies diluted in blocking solution. After washing, protein expression was analyzed by using a chemiluminescence detection kit (Thermo Scientific; 34080).

2.6 Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated using the ImProm-II Reverse Transcriptase kit (Promega, Madison, WI, http://www.promega.com; A3803) and cDNA was synthesized. Real-time PCR was performed with the CFX96 Real-Time System (Bio-Rad) and iTaq Universal SYBR Green Supermix (Bio-Rad; 172-5121). β-actin was used as the reference gene for normalization. Primers used for VCP, LC3 and p62 were as follows:

2.7 Annexin V staining and flow cytometry analysis

Cells were harvested in cold PBS and centrifuged. After discarding the supernatant, cells were resuspended and incubated in 1× Annexin-binding buffer for 15 min. Then, 5 μL of FITC Annexin V (BD Biosciences, San Jose, CA, http://www.bdbiosciences.com; 556420) was added to 100 μL of cell suspension and incubated for another 15 min. The Annexin V-stained cells were subjected to analysis using a Gallios flow cytometer (Beckman Coulter, Brea, CA, http://www.beckmancoulter.com) according to the manufacturer's instructions. Acquired data were further analyzed using the Kaluza software (Beckman Coulter).

2.8 Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) with the results of at least three independent experiments. Statistical significance was determined using the paired t-test for two-group experiments. For comparison of experiments with three or more groups, one-way analysis of variance (ANOVA) and Tukey's test were used. Differences were considered statistically significant when $p < 0.05$.

3. Result

3.1 VCP is degraded through autophagy in HCN cells following insulin withdrawal

VCP was highly expressed in adult hippocampal neural (HCN) stem cells compared to hippocampal tissue derived from 8 weeks old rat and embryonic hippocampal primary neurons (Figure 1A). The time course analysis of VCP protein and mRNA expression levels was performed following insulin withdrawal. VCP protein levels were decreased 48 h following insulin withdrawal (Figure 1B). However, the levels of VCP mRNA expression were not changed in insulin-deprived HCN cells (Figure 1C). These data suggest that VCP may be subject to post-translational regulation. VCP expression level was also significantly decreased in insulin-deprived hippocampal tissue (Figure 1D). To test whether VCP itself is a substrate for autophagic degradation or proteasome in insulin depleted HCN cells, autophagic flux inhibitor, BafA1 was treated following insulin withdrawal. Interestingly, BafA1 (30nM, 3hrs) significantly prevented VCP degradation suggesting that autophagy may be responsible for degradation of VCP in insulin-deprived HCN cells (Figure 1E).

3.2 Inhibition of VCP switched autophagic cell death to apoptosis in insulin-deprived HCN cells

VCP is known as a regulator in autophagosome maturation. We tested whether VCP ATPase activity is required for ACD. To that end, HCN cells were cultured with VCP ATPase activity inhibitor, DBeQ, in insulin-depleted media and cell death was measured (13). From here, we denote the insulin-containing and insulin-depleted culture conditions as I(+) and I(-), respectively. We tested a wide range of DBeQ concentration both in I (+) and I (-) HCN cells and chose 0.5 μM concentration of DBeQ, based on little increase of cell death in I (+) but significant increase of cell death in I (-) HCN cells (Figure 2A). Hereafter, this concentration of DBeQ has been used for our experiment. To determine cell death mode by DBeQ, the different kinds of programmed cell death inhibitors were

treated (Figure 2B). Interestingly, apoptosis inhibitor, Z-VAD-FMK significantly decreased cell death suggesting that apoptosis was induced by VCP inhibition in I(-) HCN cells, which otherwise underwent pure ACD. Further studies were performed to demonstrate the induction of apoptotic signs following VCP inhibition in I(-) HCN cells. Cleaved caspase 3 and chromatin condensation was observed with DBeQ treatment in insulin-deprived HCN cells (Figure 2D). Annexin V positive staining was also observed in DBeQ treated insulin-deprived HCN cells suggesting that inhibition of VCP switches autophagic cell death (ACD) to apoptosis in insulin-deprived HCN cells (Figure 2E).

To further demonstrate genetic inhibition of VCP also switched ACD to apoptosis, VCP siRNA was transfected. Increased cell death with VCP knockdown (KD) was confirmed (Figure 3A). An apoptosis marker, cleaved caspase3, was detected with VCP KD in HCN cells following insulin withdrawal suggesting that knockdown of VCP induces apoptosis in a similar fashion as pharmacologic inhibition of VCP (Figure 3B).

3.3 Inhibition of VCP significantly reduced autophagic flux in HCN cells following insulin withdrawal

VCP has been known toregulate autophagosome maturation in previous studies. We tested the function of VCP in ACD. VCP inhibition by DBeQ highly increased autophagic marker, type II of microtubule-associated protein 1 light chain 3 (LC3-II) in I (+) HCN cells (Figure 4A). However, LC3-II was significantly decreased in I (-) cells with VCP inhibition (Figure 4B). Decreased LC3-II can be due to high increase of autophagic flux or decrease of autophagic flux. To distinguish these two opposite possibilities, we treated HCN cells with BafA1. LC3-II was decreased in BafA1 and DBeQ treated HCN cells in I (-) compared to BafA1 treated HCN cells suggesting that inhibition of VCP decrease autophagic flux. To further demonstrate the role of VCP in autophagic flux, we used genetic inhibition of VCP. LC3-II was accumulated with VCP knockdown (KD) in I (+) condition. Besides, LC3-II was more accumulated with VCP KD and BafA1 suggesting VCP plays the role in impairment of autophagosome maturation (Figure 4C). Interestingly, LC3-II was significantly decreased with VCP

KD in I (-) condition. LC3-II was less accumulated with VCP KD and BafA1 in I (-) HCN cells (Figure 4D). To identify whether inhibition of VCP decreased LC3 mRNA expression levels, we assessed LC3 and p62 mRNA levels (Figure 4E). Expression of LC3 mRNA levels was not changed with VCP inhibition in I (-) suggesting that VCP may be a key regulator of autophagy initiation in HCN cells following insulin withdrawal.

3.4 VCP regulates autophagy initiation signaling in insulin-deprived HCN cells

Based on these results, we tested whether VCP regulates autophagy initiation signaling in insulin-deprived HCN cells. Our previous study by Ha et al., (2015) demonstrated that glycogen synthase kinase 3β (GSK-3β) induces ACD in HCN cells following insulin withdrawal. Also inactivation of GSK-3β highly decreased ACD in insulin-deprived HCN cells. Therefore, we examined whether VCP affects GSK-3β activation. Both pharmacological and genetic inhibition of VCP increased inactivation form of GSK-3β which is phosphorylated serine 9 on GSK-3β same as I (+). These data suggested that VCP may be a key regulator of autophagy initiation singling in insulin-deprived HCN cells.

4. Discussion

In this report, we demonstrated that VCP is a key regulator of autophagy initiation signaling and a mediator of crosstalk between ACD and apoptosis in insulin-deprived HCN cells. We found two novel functions of VCP by using pharmacological and genetic inhibition of VCP in HCN cells following insulin withdrawal. First, Inhibition of VCP attenuated autophagic flux in insulin-deprived HCN cells. Upon VCP inhibition, a critical upstream trigger of ACD, GSK-3β, was deactivated, as revealed by an increase of its inactivation phosphorylation on serine 9 residue, compared with insulin withdrawal alone. Second, apoptosis was induced following VCP inhibition while autophagy initiation was decreased in insulin-deprived HCN cells. Taken together, the result of our study established VCP plays an important role in autophagy initiation in insulin-deprived HCN cells.

Autophagy and apoptosis plays an essential role in development and survival of NSCs. Basal autophagy is essential to protect cells against stress conditions by degrading damaged organelles and proteins. However, excessive autophagy causes ACD. This abnormal regulation of autophagy, ACD, plays a causative role in aging and neurodegenerative diseases. The efforts of understanding the molecular mechanisms of ACD have continued in recent studies. Knowledge on the molecular mechanisms of ACD and its interconnection with apoptosis will contribute to the strategic design aimed at protecting NSCs from cell death and degenerative conditions.

Several reports implicated VCP for the autophagosome maturation step involving autophagosome and lysosome fusion. In I(+) HCN cells, VCP seems also involved in autophagosome maturation. Of note, our novel discovery indicates another function of VCP in associationwith autophagy initiation and crosstalk between autophagy and apoptosis in I(-) HCN cells, as the pharmacological inhibition and genetic suppression of VCP dramatically decreased autophagy initiation while the mode of cell death switched to apoptosis.

NSCs have attracted great attention as potential therapeutic approaches for the treatment of neurodegenerative diseases. However, the degenerating brain present adverse environment to the NSCs and causes PCD of the NSCs, greatly limiting their function. Currently, the molecular

mechanisms of PCD governing the survival and death of NSCs remain largely unknown. In HCN cells, withdrawal of insulin causes ACD even though their apoptotic machinery is intact. However, the molecular mechanisms underlying the preference of ACD over apoptosis and intera-regulation of these two pathway are just beginning to be understood. The goal of this thesis to elucidate the role of VCP in ACD and apoptosis will contribute to understanding this sophisticated molecular mechanisms of PCD in NSCs.

5. Figure legends

Figure 1. VCP is degraded through autophagy in HCN cells following insulin withdrawal

(A) VCP was abundantly expressed in HCN cells compared to hippocampal tissue and neurons. (B) VCP was significantly decreased following insulin withdrawal. (C) Expression of VCP mRNA was not changed in both I (+) and I (-). (D) VCP expression levels were decreased in I (-) *ex vivo*. (E) Degradation of VCP was prevented by BafA1 (30 nM for 3hr) in I (-).

Figure 2. Pharmacological inhibition of VCP induced apoptotic cell death in insulin-depleted HCN cells

(A) VCP inhibitor, DBeQ (0.5μM for 24hr) markedly increased cell death following insulin withdrawal. (B) Z-VAD significantly prevented cell death induced by DBeQ in I (-) and also prevented cell death induced by STS (1μM for 6 and 12hr) in I (+). (D) Apoptotic marker, cleaved caspase 3, was activated with DBeQ following insulin withdrawal. (E) Nuclear condensation was observed with DBeQ treatment. (F) Apoptosis induction was confirmed by Annexin-V staining and FACS analysis.

Figure 3. Genetic inhibition of VCP switched ACD to apoptosis

(A) Knockdown of VCP also increased apoptotic cell death in I (-) HCN cells. (B) Cleaved caspase3 was detected in VCP KD HCN cells.

Figure 4. VCP reduced autophagic flux in HCN cells following insulin withdrawal

(A) VCP inhibition induced LC3-II accumulation in I (+). (B) However, LC3-II was highly decreased in I (-). Autophagic flux was examined with BafA1. Decreased autophagic flux was confirmed in VCP inhibited I (-). (C) Knockdown of VCP accumulated LC3-II in I (+). Autophagic flux was impaired with

VCP targeting siRNA. Autophagic flux was confirmed with BafA1 in I (+). (D) However, LC3-II was markedly decreased with VCP knockdown in I (-). Autophagic flux was decreased in VCP knockdown HCN cells with BafA1 in I (-). (E) Autophagy markers, LC3 and p62, mRNA expression was not changed with VCP KD in I (-).

Figure 5. VCP regulates autophagy initiation signaling in insulin-deprived HCN cells

(A) Inactive GSK-3β (S9) was markedly recovered with DBeQ in I(-). (B) VCP knockdown increased inactive GSK-3β (S9) in I (-).

6. Figures

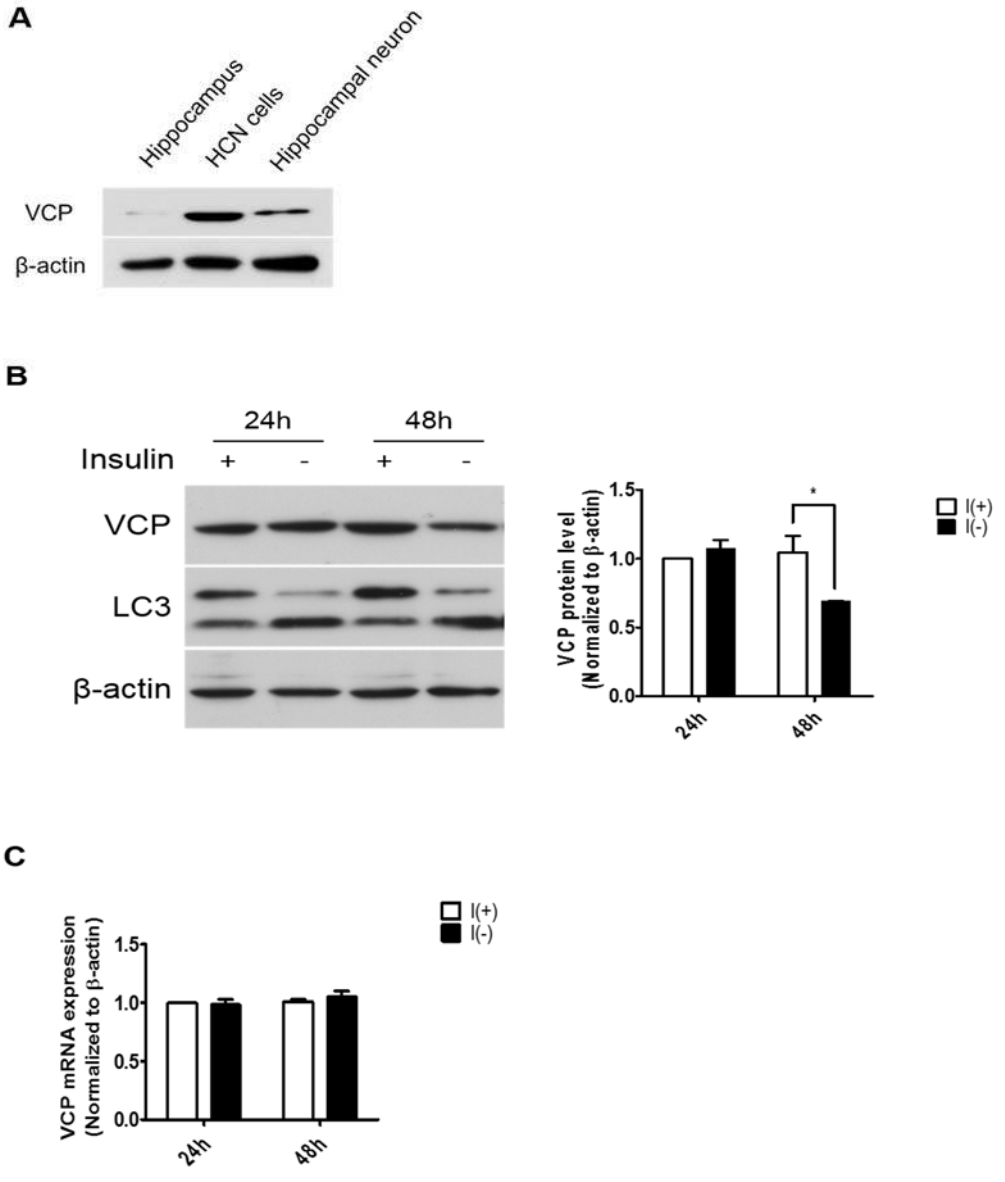
Figure 1. VCP is degraded through autophagy in HCN cells following insulin withdrawal

Figure 2. Pharmacological inhibition of VCP induced apoptotic cell death in insulin depleted HCN cells

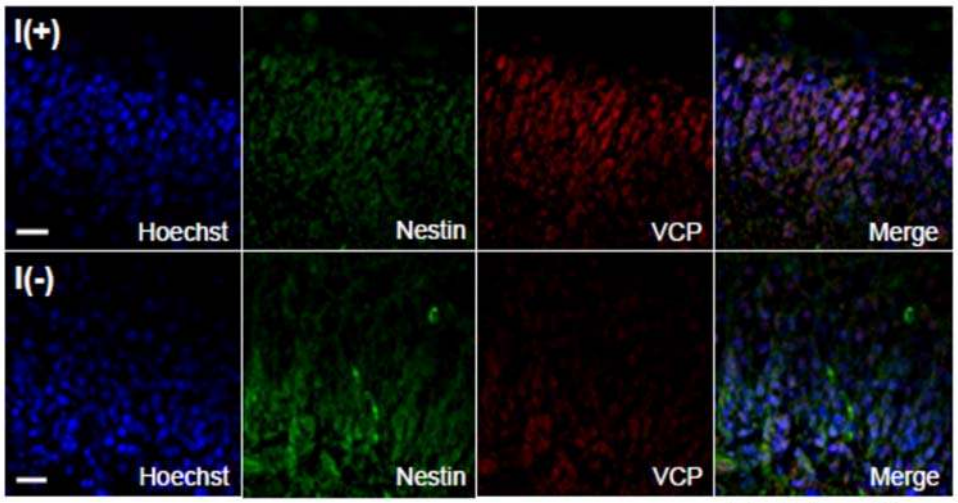
Figure 3. Genetic inhibition of VCP switched ACD to apoptosis

Figure 4. VCP reduced autophagic flux in HCN cells following insulin withdrawal

Figure 5. VCP regulates autophagy initiation signaling in insulin-deprived HCN cells


A
Hippocampus
HCN cells
Hippocampal neuron
VCP
β-actin
B
24h
48h
Insulin
+
-
+
-
VCP
LC3
β-actin
VCP protein level
(Normalized to β-actin)
1.5
1.0
0.5
0.0
*
24h
48h
I(+)
I(-)
C
VCP mRNA expression
(Normalized to β-actin)
1.5
1.0
0.5
0.0
24h
48h
I(+)
I(-)

D



* Scale bars, 20 μm

E

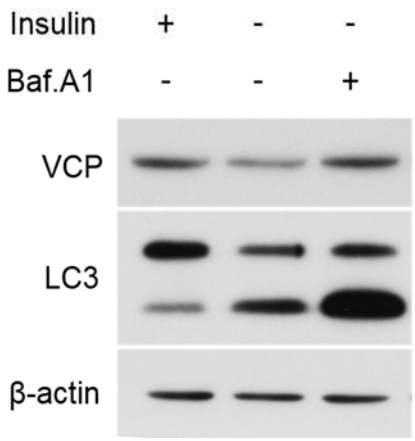
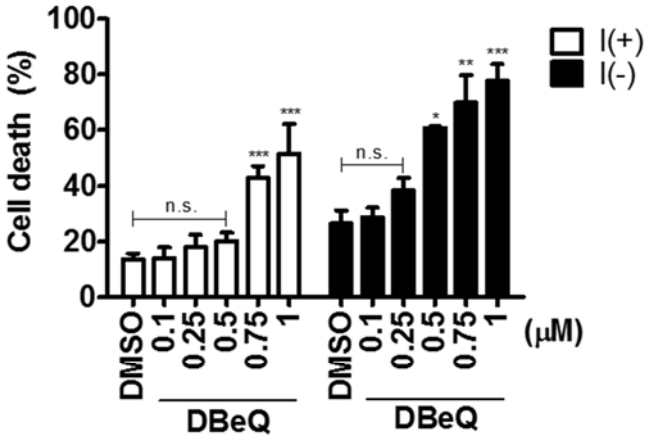
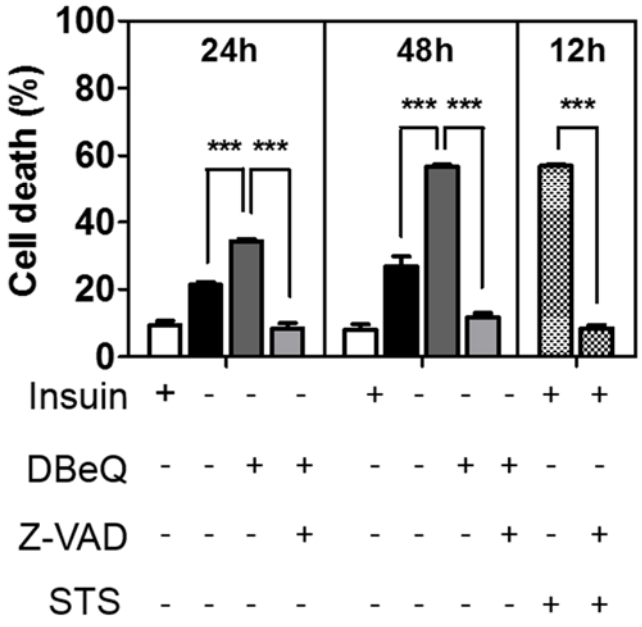


Figure 1. VCP is degraded through autophagy in HCN cells following insulin withdrawal

A


Cell death (%)
100
80
60
40
20
0
I(+)
I(-)
n.s.
n.s.
***
***
**
***
*
DMSO
0.1
0.25
0.5
0.75
1
(μM)
DBeQ
DBeQ

B


Cell death (%)
100
80
60
40
20
0
24h
48h
12h
*** ***
*** ***
***
Insuin + - - - + - - - + +
DBeQ - - + + - - + + - -
Z-VAD - - - + - - - + - +
STS - - - - - - - - + +

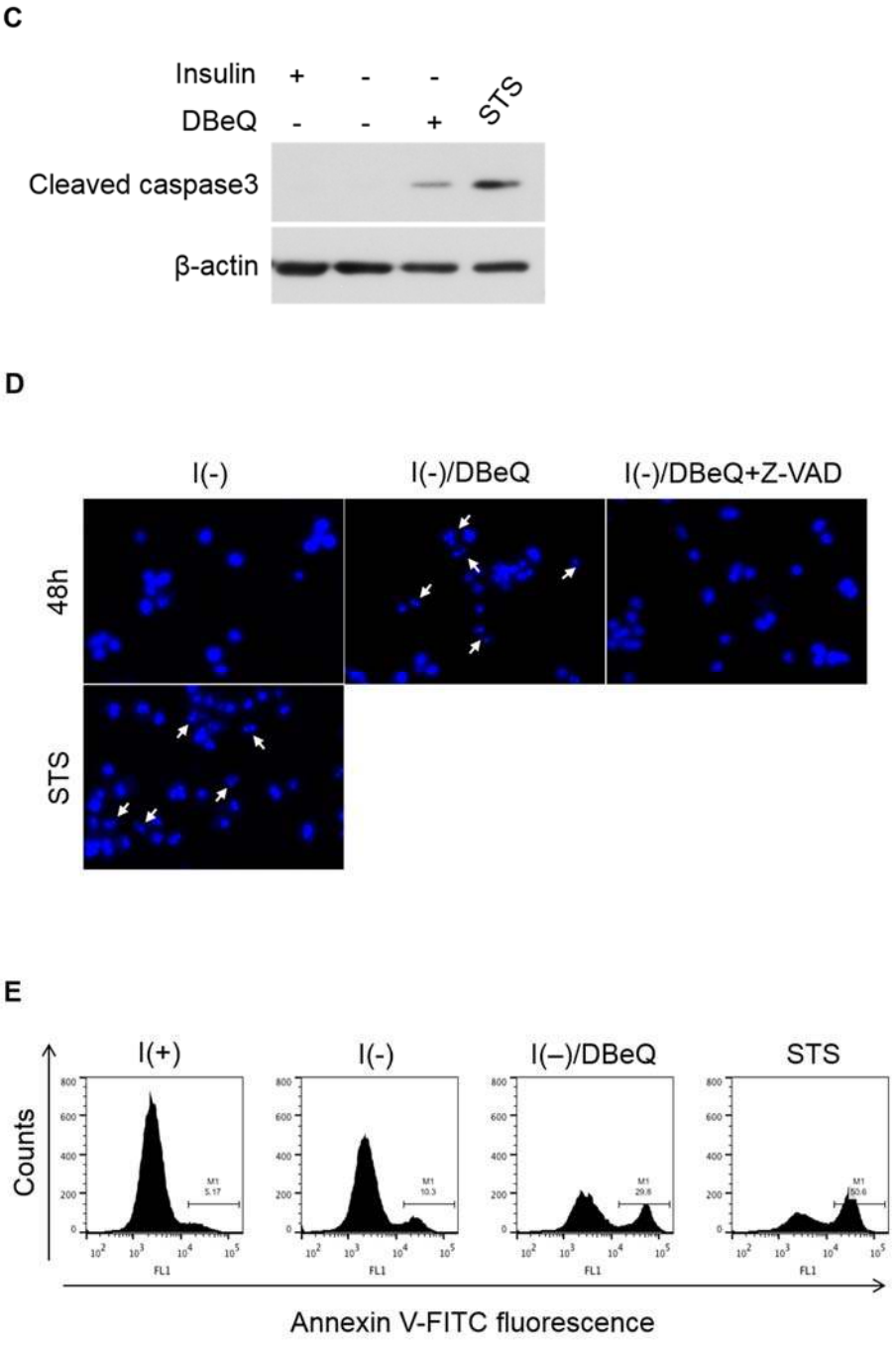


Figure 2. Pharmacological inhibition of VCP induced apoptotic cell death in insulin depleted HCN cells

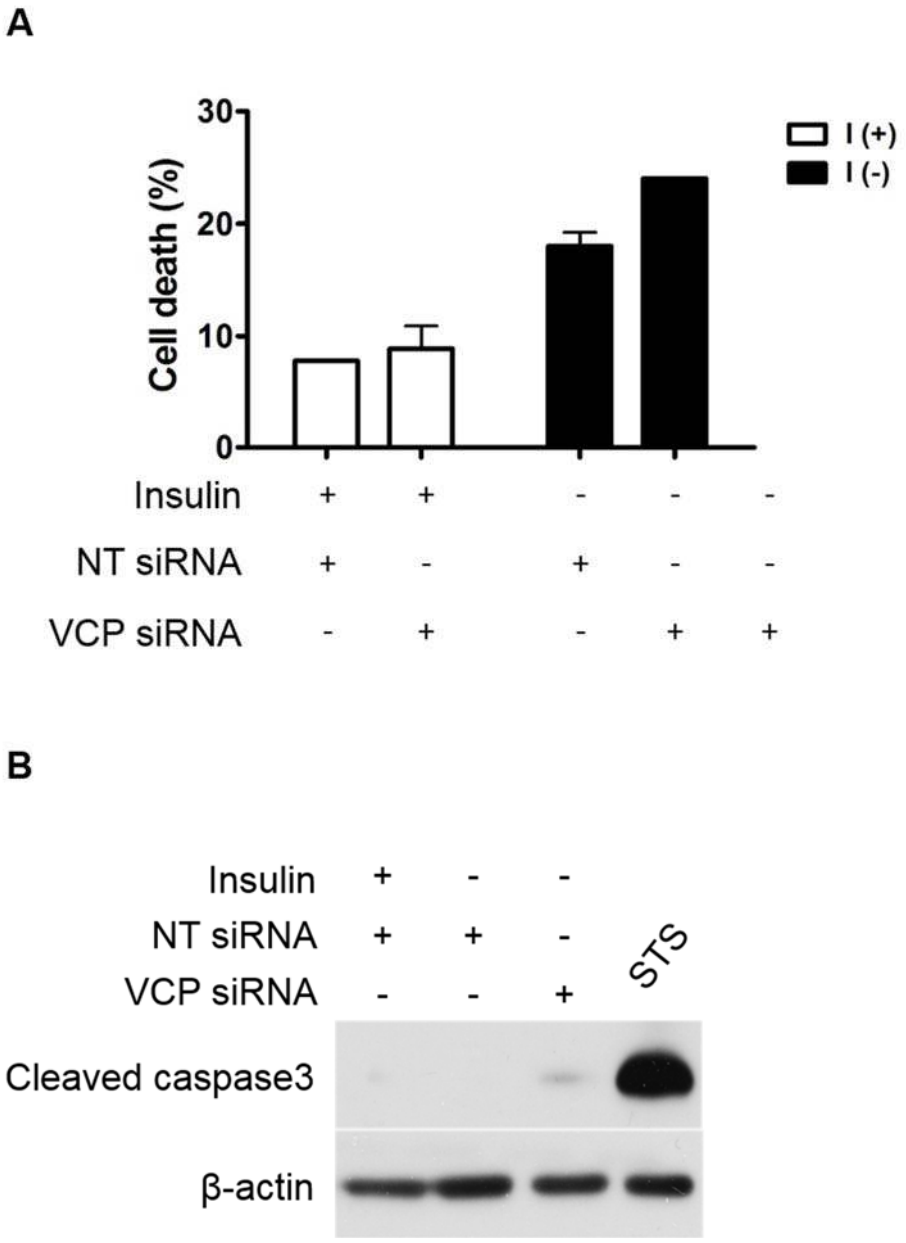
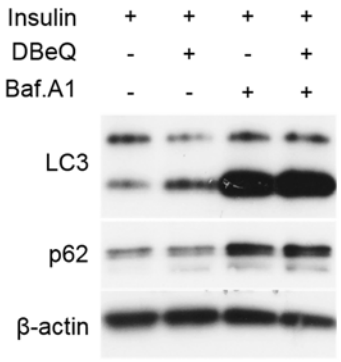


Figure 3. Genetic inhibition of VCP switched ACD to apoptosis

A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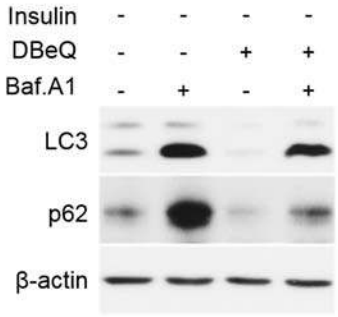
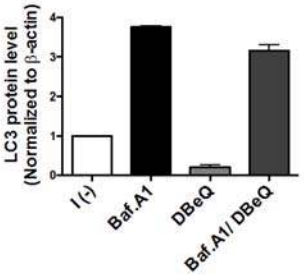
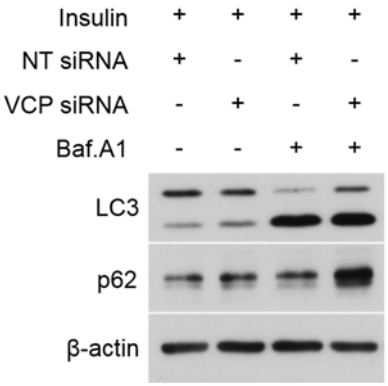
DBeQ - + - +
Baf.A1 - - + +
LC3
p62
β-actin

B


Insulin - - - -
DBeQ - - + +
Baf.A1 - + - +
LC3
p62
β-actin


LC3 protein level
(Normalized to β-actin)
0
1
2
3
4
I (-)
Baf.A1
DBeQ
Baf.A1/ DBeQ

C


Insulin + + + +
NT siRNA + - + -
VCP siRNA - + - +
Baf.A1 - - + +
LC3
p62
β-actin

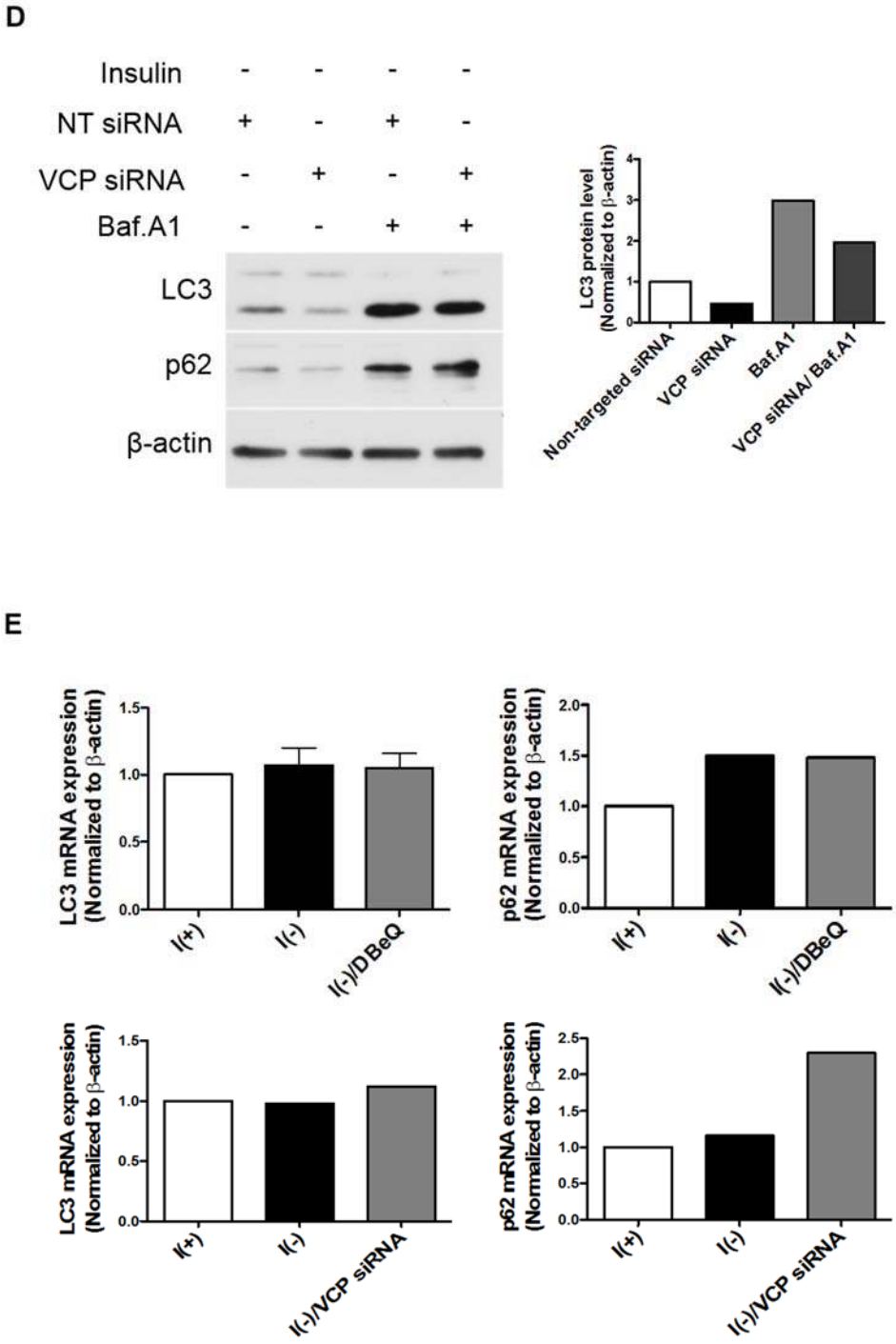


Figure 4. VCP reduced autophagic flux in HCN cells following insulin withdrawal

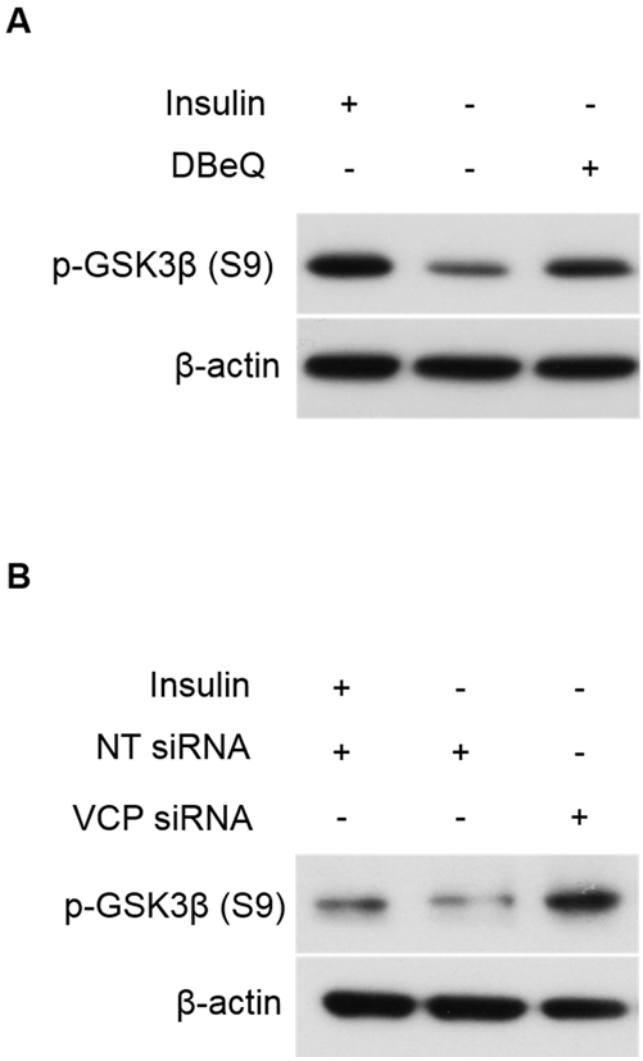


Figure 5. VCP regulates autophagy initiation signaling in insulin-deprived HCN cells

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요약문

Programmed cell death (PCD)는 신경줄기세포의 생존과 기능조절에 필수적인 역할을 한다. 따라서 신경줄기세포의 비정상적인 PCD는 노화와 퇴행성 질환에 밀접한 관련이 있다고 보고되고 있다. 그러나, 신경줄기세포의 세포사멸기작에 대한 이해는 여전히 많은 부분이 밝혀져 있지 않아신경줄기세포를 이용한 퇴행성 치료기술개발을 위한 큰 학문적 난제로 남아 있다. 그러므로, 신경줄기세포에서 일어나는 PCD의 기작을 이해 하는 것은 노화와 퇴행성 질환의 치료 탐색을 위해 중요하다. 우리는 선행연구를 통해 성인 쥐의 해마성체신경줄기세포가 인슐린 결핍 조건에서 apoptotic 대신 순수한 autophagic cell death (ACD)를 겪는다는 것을 입증하였다. 성체해마신경줄기세포는 정상적인 apoptosis 능력을 가지고 있기 때문에 인슐린이 결핍된 해마성체신경줄기세포에서왜 특이적으로 ACD가 유도되는지, 그리고 ACD와 apoptosis간의 상관관계는 어떻게 조절되는지는 아직 알려지지 않았다. Valosin-containing protein (VCP)는 포유류의 세포에서 autophagosome maturation에 필수적인 단백질이라고 알려져 있다. 본 연구에서는VCP가 인슐린이 결핍된 해마성체신경줄기세포에서 autophagic flux의 속도를 조절한다는 것을 밝혔으며, 이는 autophagosome maturation뿐만 아니라 autophagy initiation을 포함한 ACD의 다른 과정에서 VCP의 새로운 역할들을 제시한다. 특이적으로, VCP는 hippocampal tissue와 hippocampal neuron 비해 해마성체신경줄기세포에 많이 발현했다. VCP의 약리학적 유전적인 억제는 인슐린이 결핍된 해마성체신경줄기세포에서 apoptosis가 유발 되는 동안 ACD와 autophagy markers를 감소시킴을 관찰하였다. 이러한 결과들은 VCP가 인슐린이 결핍된 해마성체신경줄기세포에서 ACD 유도와 ACD와 apoptosis 사이의 상호작용 조절에 중요한 역할을 할 것이라는 것을 입증하였다. VCP가 ACD와 apoptosis간의 상호작용을 조절하는 기작을 입증하는 것은 신경줄기세포에서 PCD의 분자적 기작을 이해하는데 도움을 줄 것이라 사료된다.

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