이학석사 학위논문

AMPK-Ctbp2 상호결합이 배아줄기세포 전분화능에 미치는 영향

The effect of AMPK-Ctbp2 interaction on embryonic stem cell pluripotency

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ABSTRACT

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Stem cells undergo dynamic change of metabolic phenotype during differentiation and this feature reveals that metabolic status have pivotal role in maintain stemness. AMPK (AMP-activated protein kinase) is energy sensing kinase which is activated in energy deprivation by recognizing cellular energy status. However, that function in stem cell is not well understood. Ctbp (C-terminal binding protein) is co-repressor which regulates transcriptional activity of various gene. Previous reports reveal that Ctbp2 has a crucial role in stem cell differentiation. Furthermore, AMPK impedes Ctbp1 activity by phosphorylation. In this study, we hypothesize that in stem cells, AMPK regulates exit from pluripotency through Ctbp2 phosphorylation by energy dependent manner. First, we observed that Ctbp1 S158 residue which is phosphorylated by AMPK is conserved in Ctbp2 S164 and that residue is also phosphorylated by AMPK. Ctbp2 which is phosphorylated at S164 fails to dimerize and dissociates with NuRD complex. As a result of this, stem cell exhibits delayed differentiation. Consequently, AMPK recognizes cellular

energy state and modulates differentiation through Ctbp2. It implies that stem cells have mechanism that impede differentiation in unstable state.

Keywords: Stem cell, Energy, AMPK (AMP-activated protein kinase), Ctbp (C-terminal binding protein)

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LIST OF ABBREVIATIONS

Ctbp	C-terminal binding protein
EMT	Epithelial Mesenchymal Transition
AMPK	AMP-activated protein kinase
AMPKK	AMP-activated protein kinase kinase
LKB1	Liver kinase B1
CaMKKβ	Ca2+/calmodulin-dependent protein kinase kinase-β
AICAR	5-Aminoimidzole-4-carboxamide-1-b-D-ribofuranoside
2DG	2-deoxy-D-glucose
ESC	Embryonic Stem cell
NuRD	Nucleosome Remodeling Deacetylase
PcG	Polycomb-group

I. INTRODUCTION

1-1. Stem cell energy

Energy balance and proper metabolic status is essential for cell viability. In contrast to somatic cells, stem cells gain energy by glycolysis more than oxidative phosphorylation (Leese HJ et al., 1984). During embryonic development, they undergo dramatic change of metabolic phenotype and prefer distinct metabolites (Folmes C.D, 2012), Furthermore, in reprogramming process, metabolism shifts to glycolytic metabolism (Takahasi K et al., 2006; Folmes C.D et al., 2011a,b). In developmental process, nutrients such as glucose, pyruvate and phosphate regulate proliferation and cell fate decision (Quinn P, 1995; Conaghan J et al., 1993; Mochizuki H et al., 2011). And O-GlcNAc signaling regulates pluripotency by modifying Oct4 and Sox2 (Jang et al., 2012). In stem cell lineage specification, hematopoietic stem cells differentiate into erythroid or myelomonocytic cell fate depending on glucose and glutamin metabolism (Oburoglu L et al., 2014). Redox state also modulates differentiation ability and cell fate decision (Smith J et al., 2000; Wiley C et al., 2014; Dumollard R et al., 2007). Thus, nutrients and metabolic status are regarded to have a critical role in stemness and lineage specification although, it is not well understood.

1-2. C-terminal binding protein

Ctbp (C-terminal binding protein) was identified as phosphoprotein that binds to C-terminal region of adenovirus E1A protein and negatively regulates its activity (Schaeper U et al., 1995). Genetic analysis revealed that Ctbp acts as a transcriptional co-repressor by recognizing 'PXDLS' specific motif and interacting with transcriptional repressor such as HDAC, p300 and PC2 (Schaeper U et al., 1995; Sundqvist A et al., 1998; Koipally J et al., 2000; Sewalt RG et at., 1999). Ctbp is regarded as having a pivotal role that induces EMT (Epithelial Mesenchymal Transition), functions as antagonists of apoptosis and regulates tumorigenesis in developmental process (Grooteclaes ML et al., 2000). Furthermore, Ctbp functions as homo/hetero dimer which is mediated by NAD(+) or NADH. It is possible that Ctbp acts as cellular redox sensor (Sewalt RG et at., 1999; Thio SS et al., 2004).

Ctbp family are divided into Ctbp1 and Ctbp2. And they have highly homology. However, in developmental process, Ctbp1 deficient mice are viable but Ctbp2 deficient mice are lethal at day E10.5 (Hildebrand JD et al., 2002). In stem cell differentiation, Unlike Ctbp1, Ctbp2 levels were reduced during differentiation. And Ctbp2-knockdown ESCs exhibit delayed differentiation (Tarleton HP et al., 2010). Moreover, Ctbp2 is a component of Oct4 interactome (Esch D et al., 2013). According to a recent study, during exit from pluripotency, Ctbp2 modulates NuRD-mediated deacetylation of

H3K27 of active ESC genes and facilitates PRC-mediated H3K27me3 during ESC differentiation (Kim et al., 2015).

1-3. AMP-activated protein kinase

AMPK (AMP-activated protein kinase) is a heterotrimeric serine/threonine protein kinase that is composed of catalytic α (α1/α2) subunits and regulatory β (β1/β2) and γ (γ1/γ2/γ3) subunits. Because AMPK is activated in energy stress state, AMPK serves as energy sensor protein kinase and has an essential role in maintaining energy balance. AMPK activity is regulated by AMP/ATP ratio, Ca2+ level and NAD/NADH ratio (Carling D et al., 1989; Woods A et al., 2005; Rafaeloff-Phail et al., 2004). For activation of AMPK, kinase domain of α subunit which is located at 172 threonine is phosphorylated by AMP-activated protein kinase kinase (AMPKK) (Hawley SA et al., 1996). The major upstream kinases of AMPK are LKB1 (Liver kinase B1) and CaMKKβ (Ca2+/calmodulin-dependent protein kinase kinase-β) (Woods A et al., 2003, 2005). In energy deprived state, AMPK facilitates catabolic pathways (oxidation of fatty acids, glucose) to generate ATP and represses anabolic pathways (synthesis of lipids, glucose) to inhibit ATP consumption (Corton JM et al., 1994; Minokoshi et al., 2001; Merrill G.F et al., 1997, Muoio DM et al., 1999).

During differentiation, AMPK inhibits myoblast differentiation and negatively regulates chondrogenic differentiation (Williamson DL et al., 2009; Bandow K et al., 2014). In stem cells, AICAR (5-Aminoimidzole-4-carboxamide-1-b-D-ribofuranoside) which activates AMPK (Corton JM et al., 1995) up-regulates expression levels of pluripotency related marker genes (Adamo L et al., 2009) and sustains self-renewal ability (Shi X et al., 2013).

Furthermore, Activation of AMPK acts as metabolic barrier during reprogramming process (Vazquez-Martin et al., 2012). In cancer cell, AMPK negatively regulates Warburg effect (Faubert B et al., 2012).

1-4. Purpose

Stem cells have distinct energy metabolic phenotype compared with somatic cells (Leese HJ et al., 1984). During differentiation, stem cells undergo dynamic metabolic shifts and epigenetic change (Folmes C.D, 2012). This feature considered that stem cell energy status have relevance to the stemness.

AMPK (AMP-activated protein kinase) is cellular energy sensor kinase which is activated by energy dependent manner (Carling D et al., 1989). Several reports revealed that activation of AMPK promotes maintenance of pluripotency (Adamo L et al., 2009; Shi X et al., 2013).

Ctbp2(C-terminal binding protein2) is a transcriptional co-repressor (Schaeper U et al., 1995). It acts with epigenetic modifier such as NuRD (Nucleosome remodeling deacetylase) complex and PcG (Polycomb-group) proteins. According to a recent study, Ctbp2 have a pivotal role in early differentiation step (Kim et al., 2015).

Because AMPK impedes Ctbp1 function by phosphorylation (Kim et al., 2012) and Ctbp1, Ctbp2 have high homology, we hypothesize that AMPK retards exit from pluripotency through Ctbp2 phosphorylation by stem cell energy dependent manner.

II. MATERIALS AND METHODS

2-1. DNA constructs and site-directed mutagenesis

Flag-/Myc-Ctbp2, Flag-/HA-AMPK have been described (Kim et al., 2015; Kim et al., 2012). Mutations were introduced by site-directed mutagenesis using iPfu.

Primers for site-directed mutagenesis were listed below.

CTBP2 S164A

Sense :CAC GCG GGT TCA GGC CGT GGA GCA GAT CCG CG

Antisense : CGC GGA TCT GCT CCA CGG CCT GAA CCC GCG TG

CTBP2 S164E

Sense : CAC GCG GGT TCA GGA GGT GGA GCA GAT CCG CG

Antisense : CGC GGA TCT GCT CCA CCT CCT GAA CCC GCG TG

2-2. Antibodies and reagents

anti-Flag was purchased from Sigma-Aldrich; anti-Myc and anti-HA were obtained from Covance; anti-Ctbp2 was purchased from BD biosciences; anti-Oct4 was obtained from Santa Cruz; anti-Sox2 was purchased from R&D systems; anti-Nanog, anti-CHD4, anti-LSD1 and anti-HDAC1 were obtained

from Abcam; anti-AMPKa, anti-p-AMPKa were purchased from Cell signaling, anti-p-Ctbp2 S164 was generated from GenScript by immunization with 160-TRVQ[pS]VEQIREVAS-173.
2-DG(2-deoxyglucose) and Metformin were purchased from Sigma-Aldrich.

2-3. Cell culture

E14 mouse embryonic stem cell line was cultured on 0.1% gelatin (Sigma-aldrich) coated tissue culture dish. The medium was composed of DMEM(GE health care) and 10% (v/v) fetal bovine serum(Gibco), suppled with 100units/ml penicillin, 100μg/ml streptomycin, 2mM L-glutamin, 1% (v/v) non-essential amino acid, 55μM β-mercaptoethanol (Gipco), 30μM ciprofloxacin (Sigma-aldrich), 500units/ml ESGRO(Millipore).

HEK293FT cell line was cultured on tissue culture dish. The medium was composed of DMEM media(GE health care), 5% (v/v) fetal bovine serum and 5% (v/v) bovine calf serum, suppled with 100units/ml penicillin, 100μg/ml streptomycin.

2-4. Western blot analysis

Cells were lysed with IP Buffer(5 M Nacl, 1 M Tris-Cl(pH 8.0), 5% NP-40, 10% glycerol, 0.5 M EDTA). Lysates were separated by SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis). And transferred onto

nitrocellulose membrane for blotting using 25 mM Tris-Cl, 250 mM glycine, 15% (v/v) Methanol buffer at 4℃ for 2 hours. Membranes were blocked with 5% non-fat dry milk, 10 mM Tris-Cl(pH 7.5), 150 mM NaCl, 0.1% tween-20 for 1 hour. After blocking, membranes were washed 3 times and incubated with proper antibody at 4℃ for 16 hours. Membranes were washed 3 times and incubated with HRP(Horse radish peroxidase)-conjugated antibody at room-temperature for 1 hour. For detection, blots were reacted with ECL solution and exposured to X-ray film.

2-5. Immunoprecipitation

Co-Immunoprecipitation

Polyethylenimine (Polysciences) was used to transiently transfection to HEK293T cells. 24 hours after transfection, cells were lysis with IP150 Buffer (5M Nacl, 1M Tris-Cl(pH 8.0), 5% NP-40, 10% glycerol, 0.5M EDTA). Lysate incubate with 1μg of proper antibody and protein A/G bead at 4℃ for 2hours.

Endo-Immunoprecipitation

E14 or HEK293T cells were lysed with IP Buffer (5 M Nacl, 1 M Tris-Cl (pH 8.0), 5% NP-40, 10% glycerol, 0.5 M EDTA). Lysates were incubated with 1 μg of proper antibody and protein A/G bead at 4℃ for 2 hours.

2-6. RNA isolation

Total RNA were extracted with Trizol (Invitrogen). Cell extracts mixed with chloroform and incubated at room temperature for 5 min. Superannuates incubate with iso-propanol at room temperature for 10 min. Centrifuge 12000g for 15 min. Pellet was washed by 75% ethanol in DEPC.

2-7. Quantitative real-time PCR

cDNA was synthesized using AMV Reverse Transcriptase(Sigma-Aldrich). cDNA was diluted to proper concentration and mixed with SYBR premix Ex Taq (Takara). Reaction was processed by manufacture's protocol.

Primers for qRT-PCR are listed in table 1.

2-8. Protein purification

For protein purification, GST-fused constructs were cloned into pGEX 4T-1 vector and His-fused constructs were cloned into pRSET vector. GST-fusion protein and His-fusion protein were expressed in BL21. 1 mM IPTG was used for protein induction at 37℃ for 3 hours. E.coli were suspended using STE buffer (10 mM Tris-Cl (pH 7.4) , 1 mM EDTA, 160 mM NaCl) and add 0.1 M PMSF, 0.1 g/ml lysozyme (sigma). After incubation, 1% (v/v) Protease

inhibitor cocktail (GenDEPOT) and 1% (W/v) sarcosine were added. Sonication was performed and incubated with 1% (v/v) Triton X-100. GST or His-fusion protein were purified using glutathione sepharose 4B bead(GE health care) or Ni-NTA agarose bead (Qiagen) respectively. GST-fusion protein were eluted using 20 mM reduced glutathione, 100 mM Tris-Cl (pH8.0), 120 mM NaCl, 10% glycerol. And His-fusion protein were washed with 10 mM imidazole and were eluted using 150 mM imidazole, 160 mM NaCl, 100 mM Tris-Cl (pH7.4), 10% glycerol.

2-9. GST-pull down assay

GST-fusion protein were incubated with cell lysates or His-fusion protein with binding buffer (5 M Nacl, 1 M Tris-Cl (pH 8.0), 5% NP-40, 10% glycerol, 0.5 M EDTA) at 4℃ for 4 hours. Next, GST-fusion protein were purified using glutathione sepharose 4B beads (GE health care).

2-10. Lenti virus-based shRNA

pLKO.1 puro vectors are purchased from Sigma-Aldrich for expressing shRNAs. PLP1 0.5 μg, PLP2 0.5 μg, VSVG 0.5 μg and 0.5 μg of shRNA were transfected to HEK293FT cells by Lipofectamine plus (Introgen). After 48 hours, media which were contained viruses were filtrated by 0.45 μM filter

(Sartorius AG). Cells were infected by virus which were mixed with media 1:1 as ratio with 1 μg/ml polybrene.

shRNAs used for gene knockdown are listed below

Human Ctbp2 shRNA(TRCN0000109335, TRCN000307495),

2-11. Self-Renewal Assay

ESCs were trypsinized to single cell suspension and cultured 500 cells/well in 6 well plate. Cells were incubated with media which is supplied with 0/5/500 U LIF during 7 days. Alkaline phosphates are stained with Fast Red Violet (Sigma-Aldrich) and Naphthol AS-BI (Sigma-Aldrich).

2-12. In vitro kinase assay

AMPKa (Millipore) was incubated with GST-Ctbp2 in reaction buffer (15 mM HEPES, 6.25 mM β-glycerol phosphate, 1.25 mM EGTA, 0.45 mM dithiothreitol, 18.75 mM MgCl2, 125 μM ATP, 150 μM AMP, 10 μCi 32P-ATP) at 30℃ for 1 hour. Proteins were separated by SDS-PAGE. Gel was dried at 80℃ for 1 hour and exposured to X-ray film.

	Gene	5’ primer	3’ primer
Real-time PCR	18s	TTAGAGTGTTCAAAGCAGGC CCGA	TCTTGGCAAATGCTTTCGCT CTGG
	Oct4	GGCGTTCTCTTTGGAAAGGT GTTC	CTCGAACCACATCCTTCTCT
	Sox2	GAGAGCAAGTACTGGCAAGA CCG	ATATCAACCTGCATGGACAT TTTT
	Nanog	ATGAAGTGCAAGCGGTGGC AGAAA	CCTGGTGGAGTCACAGAGT AGTTC
	Klf2	CGCACCTAAAGGCGCATCTG	TTCGGTAGTGGCGGGTAAG C
	Klf4	ACAGGCGAGAAACCTTACCA CTGT	GCCTCTTCATGTGTAAGGCA AGGT
	Nrob1	TCCAGGCCATCAAGAGTTTC	ATCTGCTGGGTTCTCCACTG
	Esrrb	TGCACACAGTTCCTTCCAAA TGGC	AGCTTGGGCCACCATGTCAT CTAT
	Nr5a2	AACCCTATTGCCAGCATCTT TGCC	GTGCTCGTTCAGCTTCACAT TGCT
	Fgf4	CTCTTCGGTGTGCCTTTCTTT ACCGA	AGGAAGGAAGTGGGTTACC TTCATGG
	Tdh	GGACAATGTGATCCTATCAG ACATA	CTCACGGAGGCTCTTGTAAT C
	Zfp42	CAGCTCCTGCACACAGAAGA	ACTGATCCGCAAACACCTG

Table 1. qRT-PCR primer list

Ⅲ. RESULTS

3-1. AMPK activation retards exit from pluripotency during ESC differentiation.

First, to investigate the molecular function of AMPK during ESC differentiation, we treated 2DG (2-deoxy-D-glucose) or metformin which are known to activate AMPK(Wang et al., 2011; Zhou et al., 2001). In ESCs, both drugs effectively activate AMPK (Figure 3-1-1., Figure 3-1-2.). To determine that activated AMPK by 2DG or metformin influences differentiation in ESCs, we performed self-renewal assay treated with 2DG or metformin dose-dependent manner. 2DG or metformin treated ESCs exhibited sustained alkaline phosphatase activity at 1 mM, 10 mM respectively without affecting proliferation and morphology (Figure 3-1-3., Figure 3-1-4.). Consistent with above results, we observed that differentiated ESCs that were treated with 2DG or metformin exhibited less reduced mRNA expression level of pluripotency related marker genes by qRT-PCR (Figure 3-1-5., Figure 3-1-6.). Consequently, these data suggested that activated AMPK by 2DG or metformin retards exit from pluripotency during ESC differentiation.

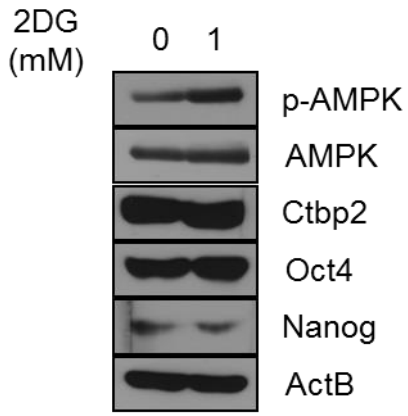

Figure 3-1-1. 2DG activates AMPK in ESCs.

Increased p-AMPK level in E14 cells is detected by western blot analysis upon treatment of 2DG 0 or 1mM for 1hour. AMPK level is not changed. ActB is used as control.

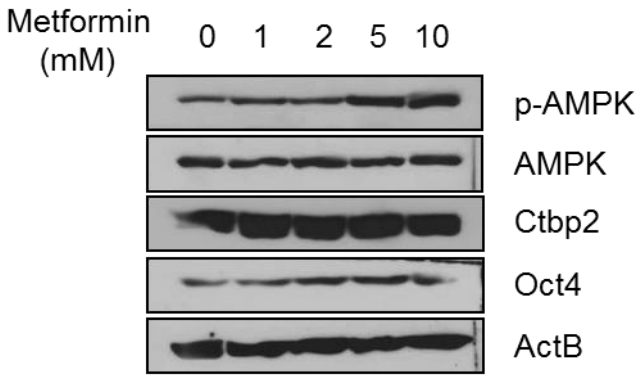

Figure 3-1-2. Metformin activates AMPK in ESCs.

Increased p-AMPK level in E14 cells is detected by western blot analysis upon treatment of metformin indicated concentration for 2hours.

AMPK level is not changed. ActB is used as control.

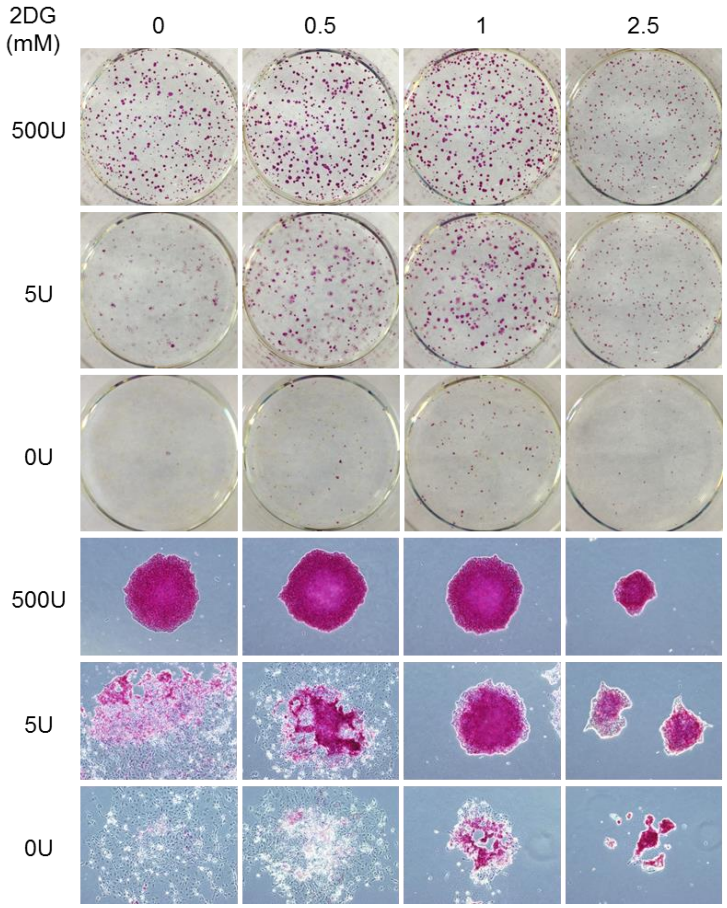

Figure 3-1-3. 2DG retards exit from pluripotency during ESC differentiation (1).

E14 cells cultured in 0/5/500U LIF condition with 2DG as indicated concentration during 7 days. The undifferentiated state was evaluated by AP staining and morphology.

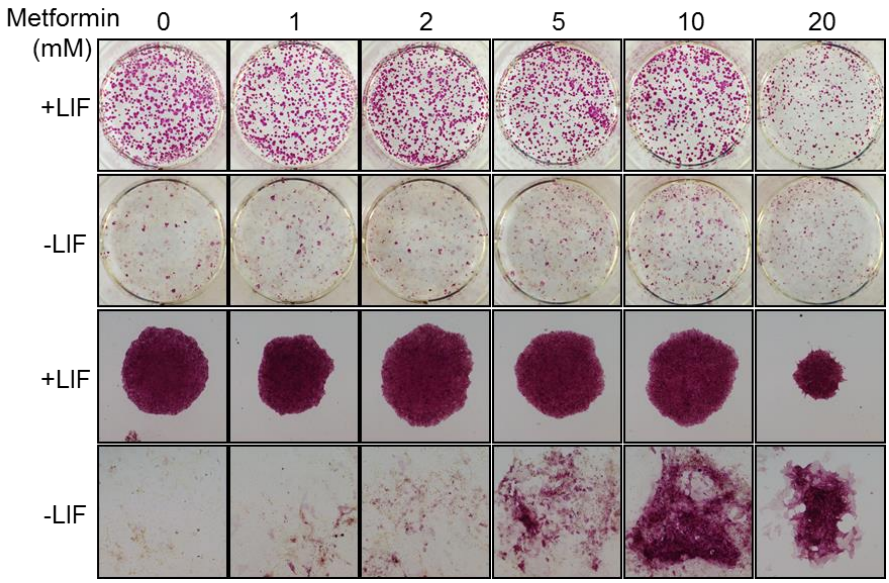

Figure 3-1-4. Metformin retards exit from pluripotency during ESC differentiation (1).

E14 cells cultured in 0/5/500U LIF condition with metformin as indicated concentration during 7 days. The undifferentiated state was evaluated by AP staining and morphology.

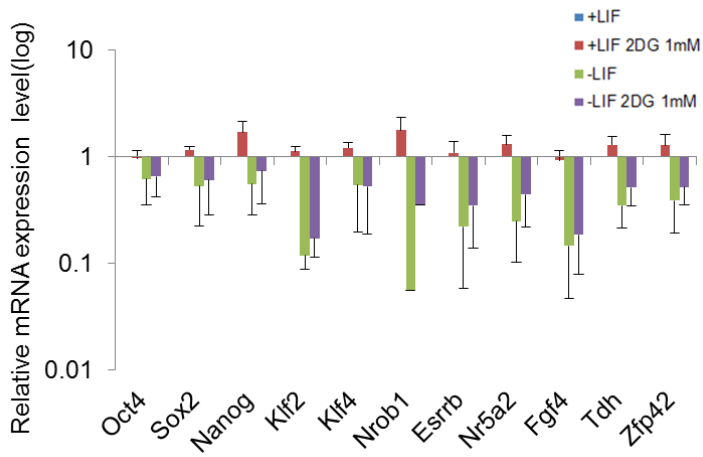

Figure 3-1-5. 2DG retards exit from pluripotency during ESC differentiation (2).

Quantitative real-time PCR result showing that mRNA expression levels of pluripotency-related genes(Oct4, Sox2, Nanog, Klf2, Klf4, Nrob1, Esrrb, Nr5a2, Fgf4, Tdh, Zfp42) reduced during differentiation. 2DG treated E14 shows partially delayed differentiation levels.

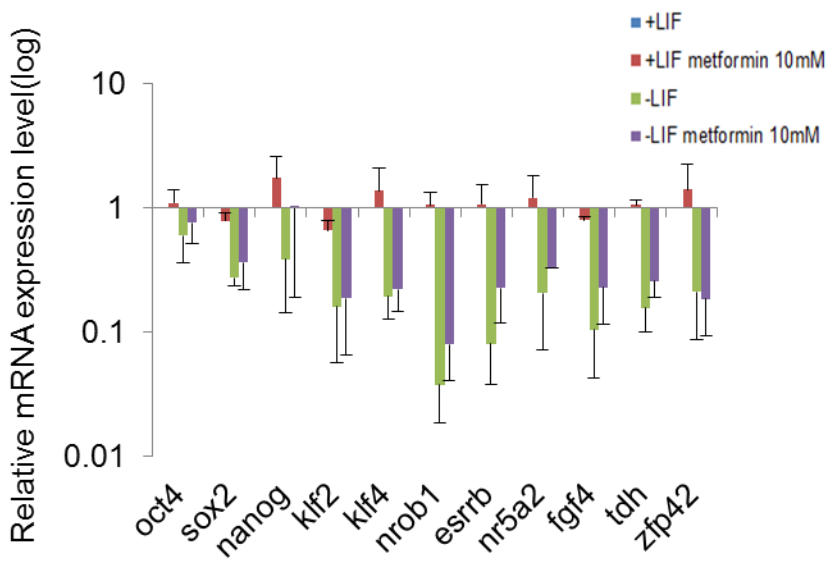

Figure 3-1-6. Metformin retards exit from pluripotency during ESC differentiation (2).

Quantitative real-time PCR result showing that mRNA expression levels of pluripotency-related genes(Oct4, Sox2, Nanog, Klf2, Klf4, Nrob1, Esrrb, Nr5a2, Fgf4, Tdh, Zfp42) reduced during differentiation. Metformin treated E14 shows partially delayed differentiation levels.

3-2. AMPK interacts with Ctbp2 and phosphorylates at S164 in ESCs.

We previously reported that Ctbp2 is essential for exit from pluripotency during ESC differentiation and AMPK phosphorylates Ctbp1 at S158 and regulates its activity (Kim et al., 2015; Kim et al., 2012). Based on the previous data that AMPK is a component of Ctbp2 complex (Figure 3-2-1.) (Kim et al., 2015), first, to determine whether AMPK phosphorylates Ctbp2 in ESCs, we examined physical interaction between AMPK and Ctbp2. Flag-CTBP2 and HA-AMPK were transiently transfected to HEK293T cells and lysate were precipitated with anti-Flag antibody. We observed AMPK wild type a1 binds to Ctbp2. AMPK active a1 and a2 also bind to Ctbp2 (Figure 3-2-2.). In stably Flag-CTBP2 overexpressed ESCs, we also observed that CTBP2 interacts with endogenous AMPK by immuno-precipitation (Figure 3-2-3.).

Because AMPK phosphorylates serine 158 residue of Ctbp1 and that was well conserved in Ctbp2 at S164 (Figure 3-2-4.), we investigated whether AMPK phosphorylates Ctbp2 at S164. First, we substituted CTBP2 at 164 serine to alanine (Figure 3-2-5.). To investigate whether CTBP2 is phosphorylated at 164 serine residue by AMPK, we performed in vitro kinase assay using [32P]-ATP. Compared with wild type CTBP2, S164A mutant exhibited reduced phosphorylation level (Figure 3-2-6.). To confirm this result, we generated anti-p-Ctbp2 S164 antibody. We tested that this antibody detects

phospho-peptide but not non-phospho-peptide by dot blot and peptide competition assay (Figure 3-2-7.). Using this antibody, we observed that phosphorylation level of CTBP2 was increased depending on amounts of AMPK (Figure 3-2-8.). Contrarily, CTBP2 S164A mutant was not phosphorylated (Figure 3-2-9.). In line with above data, 2DG or metformin treated cell lysate incubated with CTBP2 exhibits increased phosphorylation level than non-treated control cell and it was also observed in active form of AMPK a1 or a2 overexpressed ESCs (Figure 3-2-10., Figure 3-2-11.). These data indicated that AMPK interacts with Ctbp2 and phosphorylates at S164 in ESCs..

Protein name	Accession number	Mock		Ctbp2	
		1' trial	2' trial	1′ trial	2' trial
AMPK alpha-1	IPI00556823	0	0	16	13
AMPK alpha-2	IPI00123445	0	0	3	0
AMPK beta-1	IPI00223185	0	0	8	9
AMPK gamma-1	IPI00119930	0	0	16	12
AMPK gamma-2	IPI00128126	0	0	2	2

Figure 3-2-1. AMPK is a component of Ctbp2 complex..

Ctbp2 complex data is shown. AMPK is a component of Ctbp2 complex.

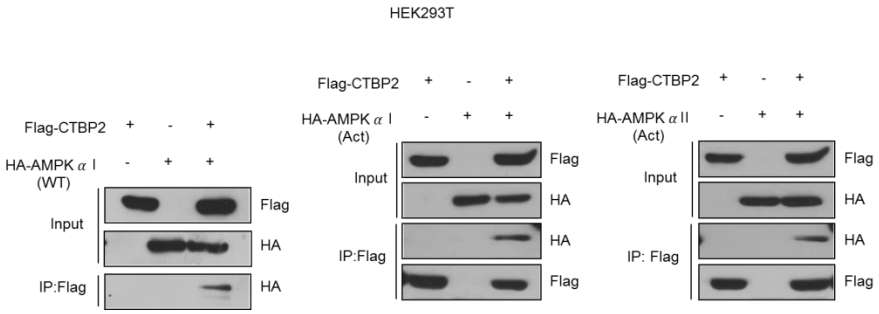

Figure 3-2-2. AMPK binds to Ctbp2 in HEK293T.

Flag-CTBP2 and HA-AMPK constructs were transiently co-transfected in HEK293T cells. Immunoprecipitation was used by anti-Flag antibody.

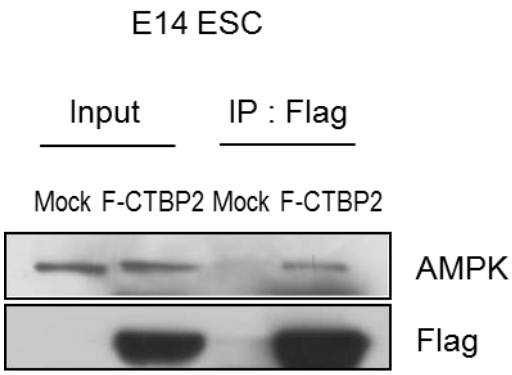

Figure 3-2-3. AMPK binds to Ctbp2 in ESCs.

Flag-CTBP2 overexpressed E14 cells were lysed and immunoprecipitated with Anti-Flag bead(M2). Mock was used as negative control.

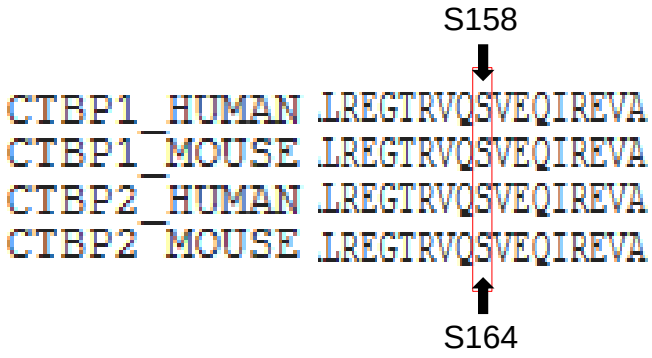

Figure 3-2-4. AMPK phosphorylation residue is well conserved in Ctbp.

Sequence alignment result between human, mouse Ctbp1 and human, mouse Ctbp2. AMPK phosphorylation residue is highlighted by red box.

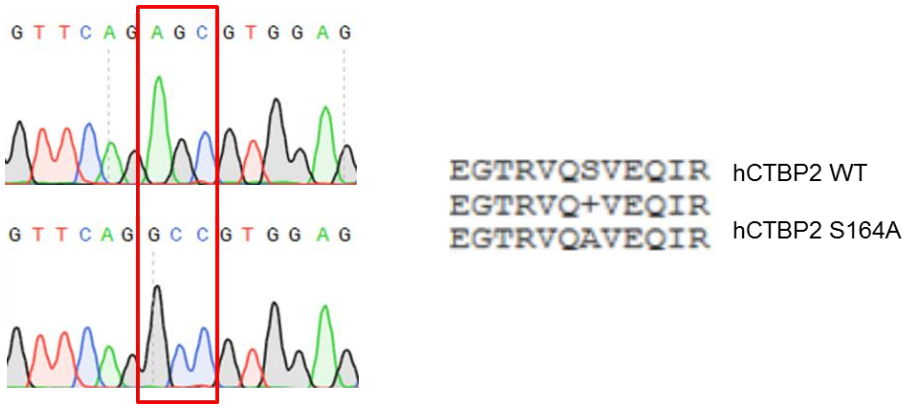

Figure 3-2-5. Sequencing result of Ctbp2 S164A mutant.

Sequencing result of Ctbp2 S164A mutant. Nucleotides substituted AGC to GCC. Amino acid substituted Serine to Alanine.

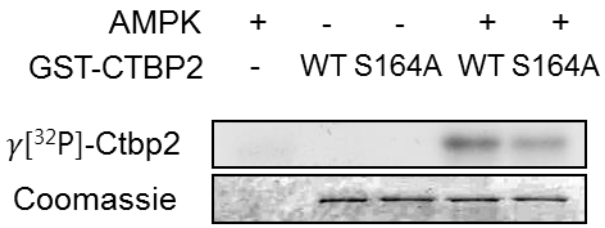

Figure 3-2-6. AMPK phosphorylates Ctbp2 at S164 (1).

In vitro kinase assay data indicated that compared with WT CTBP2, S164A mutant CTBP2 exhibits reduced phosphorylation levels. AMPK incubated with GST-CTBP2 protein and [^{32}P]-ATP. Coomassie staining showing that same amounts of wildtype and mutant proteins.

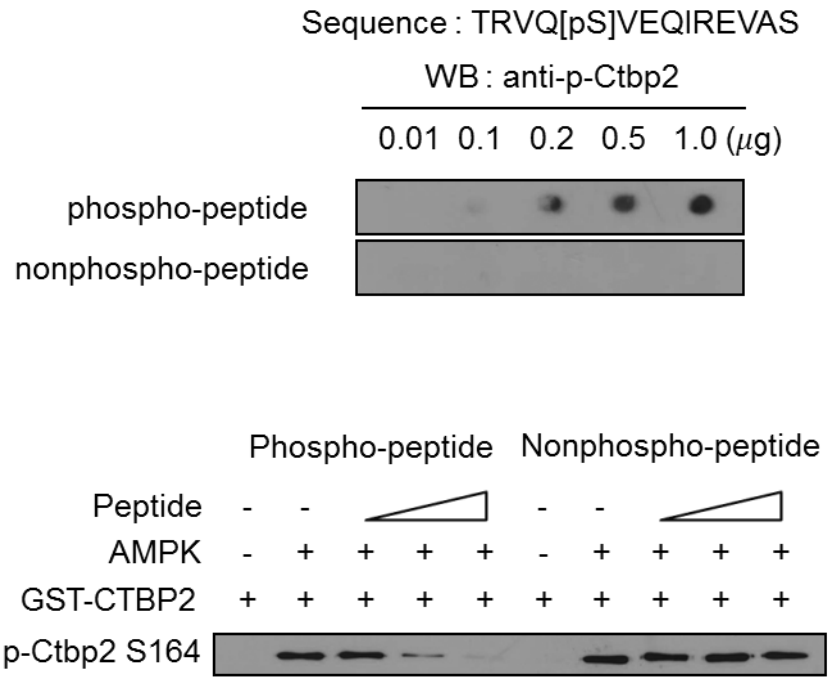

Figure 3-2-7. Anti-phospho-Ctbp2 S164 antibody detects phosphorylated Ctbp2 at S164.

Dot blot assay was performed using phospho-peptide or non-phospho-peptide indicated concentration. Blot detected by customized anti-phospho-Ctbp2 S164 antibody. AMPK incubated with GST-CTBP2 protein before peptide-competition assay. To competition, anti-phospho-Ctbp2 antibody was incubated with phospho-peptide or non-phospho-peptide(1/5/10nM).

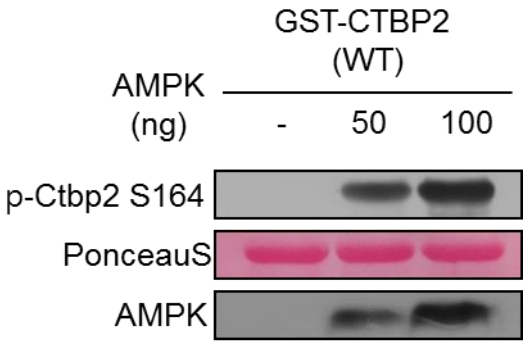

Figure 3-2-8. AMPK phosphorylates Ctbp2 at S164 (2).

Western blot analysis shows that increased phospho-Ctbp2 S164 levels depending on amounts of AMPK. For detects phospholyated-CTBP2 blot, anti-phosho-Ctbp2 S164 antibody was used. PonceauS staining shows same amounts of GST-CTBP2.

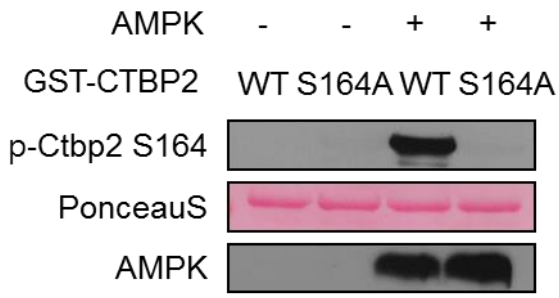

Figure 3-2-9. AMPK phosphorylates Ctbp2 at S164 (3).

Western blot analysis shows that AMPK phosphorylates S164 of CTBP2 wildtype but not S164A mutant CTBP2. For detects phospholyated-CTBP2 blot, anti-phosho-Ctbp2 S164 antibody was used. PonceauS staining shows same amounts of GST-CTBP2.

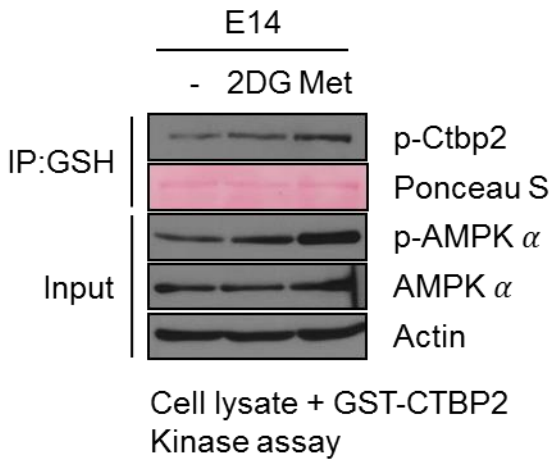

Figure 3-2-10. 2DG and Metformin induce Ctbp2 at S164 phosphorylation.

GST-CTBP2 incubated with 2DG or metformin treated E14 lysates. Lysates precipitate with glutathione sepharose beads. For detects phospholyated-CTBP2 blot, anti-phosho-Ctbp2 S164 antibody was used. PonceauS staining shows same amounts of GST-CTBP2.

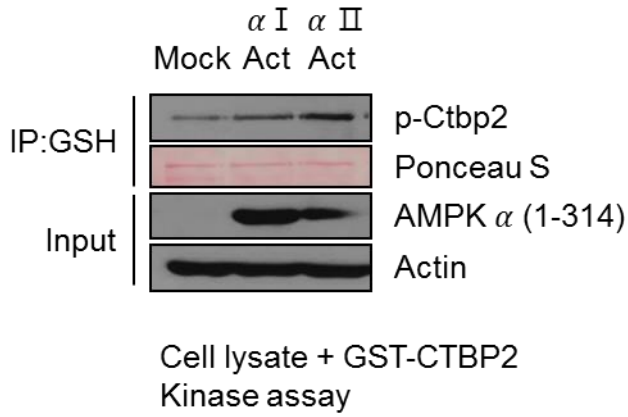

Figure 3-2-11. AMPK a1 and a2 phosphorylates Ctbp2 at S164.

GST-CTBP2 incubated with AMPK activated E14 lysates. Lysates precipitate with glutathione sepharose beads. For detects phospholyated-CTBP2 blot, anti-phosho-Ctbp2 S164 antibody was used. PonceauS staining shows same amounts of GST-CTBP2.

3-3. Phosphorylated Ctbp2(S164E) dissociates with NuRD complex and leads to incomplete exit from pluripotency during differentiation.

Next, To determine role of phosphorylation of Ctbp2 at S164, we substituted at 164 serine to glutamic acid (phospho mimic form) (Figure 3-3-1.), Wild type and S164E mutant Flag-CTBP2 were rescued in Ctbp2-knockdown ESCs (Figure 3-3-2.), As expected, we observed that wild type CTBP2 rescued shCtbp2 ESCs re-obtained pluripotency. Whereas S164E mutant CTBP2 rescued shCtbp2 ESCs exhibited incomplete exit from pluripotency by AP-staining, qRT-PCR and western blot assay (Figure 3-3-3., Figure 3-3-4., Figure 3-3-5.).

Because Ctbp2 functions as home/hetero dimer (Sewalt RG et at., 1999), we postulated that unlike wild type, S164E mutant rescued shCtbp2 ESCs which failed to exit from pluripotency is due to the fact that phosphorylation of S164 inhibits formation of Ctbp2 dimer. To test this hypothesis, we performed dimerization assay. As expected, it was observed that S164E CTBP2 loses their ability to dimerize by immunoprecipitation in HEK 293T (Figure 3-3-6.). And we confirmed this data by in vitro binding assay using GST-/His-CTBP2 (Figure 3-3-7.). To confirm this results are not due to artificial effect of mutant formation, we performed in vitro kinase assay using serially increase amounts of AMPK and verified that CTBP2 loses its ability to dimerize depending on S164 phosphorylation level (Figure 3-3-8). Furthermore, Ctbp2 functions with NuRD complex which represses pluripotency gene expression (Reynolds et al., 2012; Kim et al., 2015). To determine the effect of phosphorylation of

serine at 164 with regard to NuRD complex, we test interaction between CTBP2 and NuRD complex. Compared with wild type, S164E CTBP2 exhibits reduced binding ability in HEK293T and ESCs (Figure 3-3-9., Figure 3-3-10.). This was also confirmed by GST-pull down assay (Figure 3-3-11). In consequence, AMPK phosphorylates Ctbp2 at S164 and phosphorylated Ctbp2 dissociates with NuRD complex and leads to incomplete exit from pluripotency during differentiation (Figure 3-3-12.).

Figure 3-3-1. Sequencing result of Ctbp2 S164E mutant.

Sequencing result of Ctbp2 S164E mutant. Nucleotides substituted AGC to GAG. Amino acid substituted Serine to Glutamic acid.

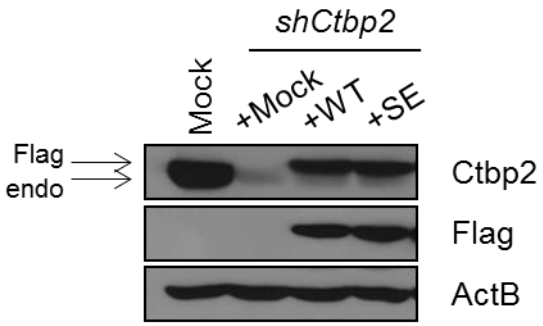

Figure 3-3-2. Rescue of CTBP2 wildtype and S164E mutant.

Rescue of wildtype and S164E mutant Ctbp2 in mCtbp2-knockdown E14 cells was detected by western blot analysis. Arrows indicates ectopic CTBP2 and endo Ctbp2.

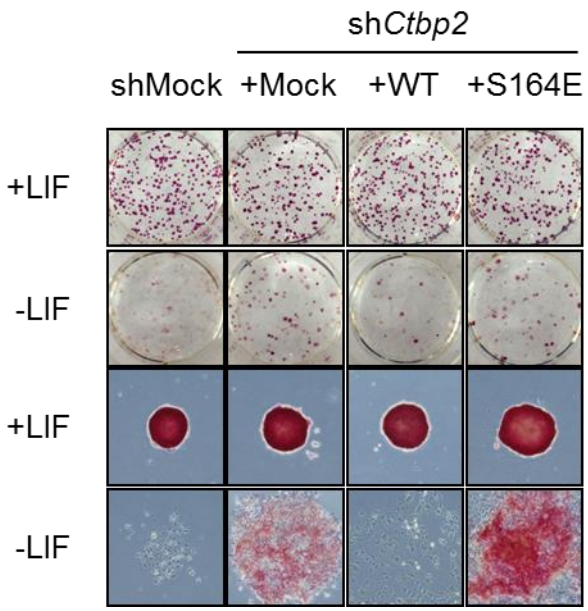

Figure 3-3-3. CTBP2 S164E mutant leads to incomplete exit from pluripotency during differentiation (1).

AP staining results indicate that knockdown of mCtbp2 leads to incomplete exit from pluripotency. Wildtype CTBP2 rescued E14 normally differentiated but, S164E mutant CTBP2 rescued E14 cells exhibits delayed differentiation.

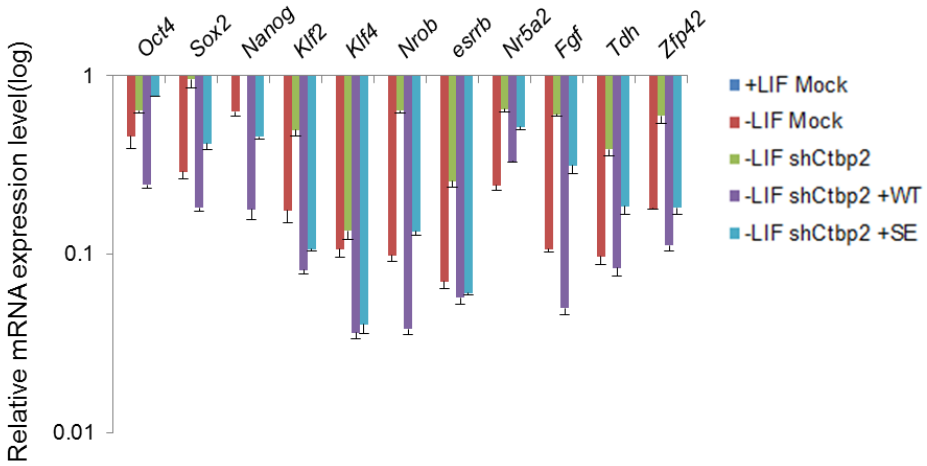

Figure 3-3-3. CTBP2 S164E mutant leads to incomplete exit from pluripotency during differentiation (1).

Quantitative real-time PCR result showing that pluripotency related mRNA expression levels of S164E mutant CTBP2-recued ESCs partially sustained compared with wildtype CTBP2-rescued ESCs during differentiation.

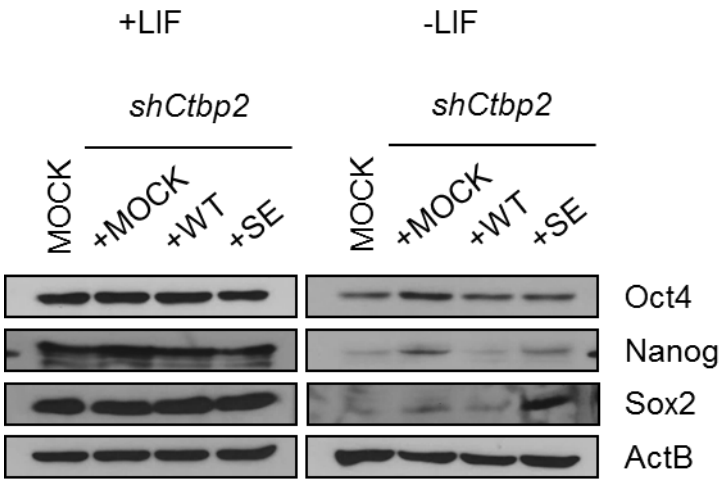

Figure 3-3-5. CTBP2 S164E mutant leads to incomplete exit from pluripotency during differentiation (3).

In western blot analysis, S164E mutant CTBP2-recued ESCs sustain Oct4, Nanog and Sox2 protein levels compared with wildtype CTBP2-rescued ESCs. ActB was used as control.

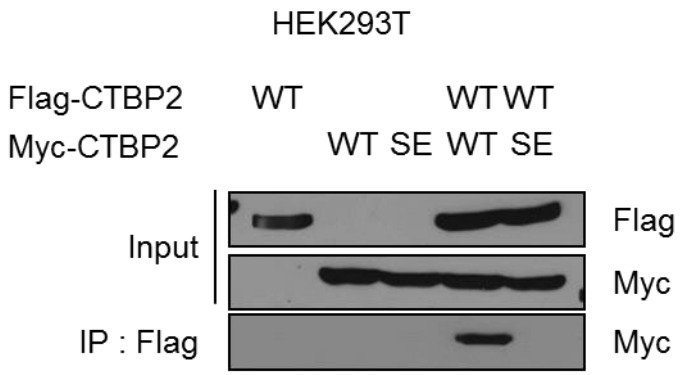

Figure 3-3-6. CTBP2 S164E mutant fails to dimerize with CTBP2 wildtype in HEK293T.

Flag-CTBP2 and Myc-CTBP2 were transiently co-transfected to HEK293T cells. Anti-Flag antibody used for precipitation. While wildtype CTBP2, S164E mutant CTBP2 fail to dimerize.

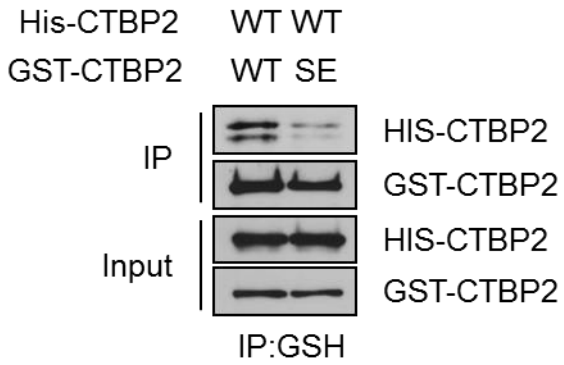

Figure 3-3-7. GST-CTBP2 S164E mutant fails to dimerize with HIS-CTBP2 wildtype.

His-CTBP2 protein incubate with wildtype or S164E mutant GST-CTBP2. Glutathione sepharose bead was used for precipitation. Compared with wildtype, S164E mutant exhibits reduced binding ability.

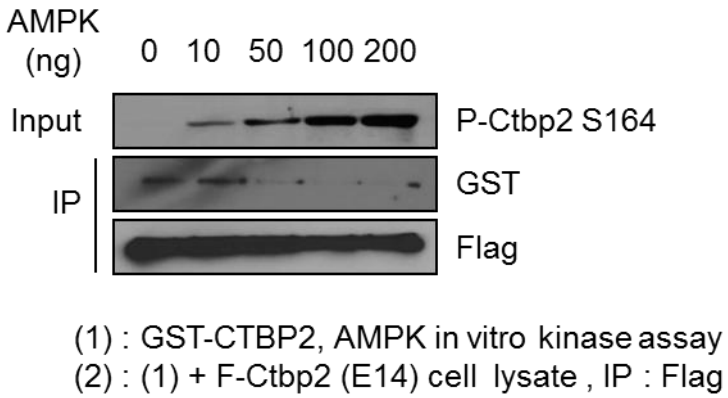

Figure 3-3-8. Phosphorylation of CTBP2 at S164 by AMPK inhibits CTBP2 dimerization..

GST-CTBP2 incubate with AMPK as indicated concentration. Phosphorylated CTBP2 reduced their ability to dimerize. Phospho-Ctbp2 was detected by anti-phospho-Ctbp2 S164 antibody and precipitation was performed by glutathione sepharose beads.

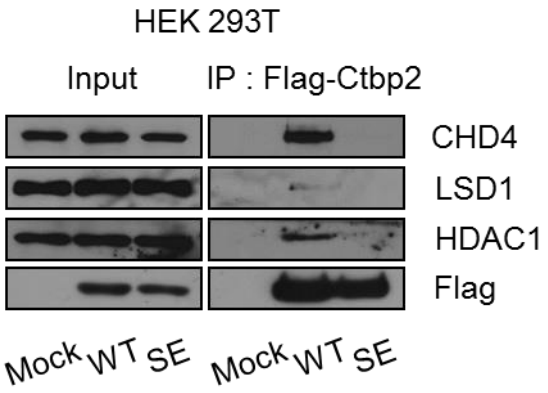

Figure 3-3-9. CTBP2 S164E mutant dissociates with NuRD complex in HEK293T.

Flag-CTBP2 wildtype and S164E mutant transiently transfected to HEK293T cells. Wildtype CTBP2 binds to endogenous CHD4, LSD1 and HDAC1. Compare with wildtype, S164E mutant Ctbp2 exhibits reduced binding ability.

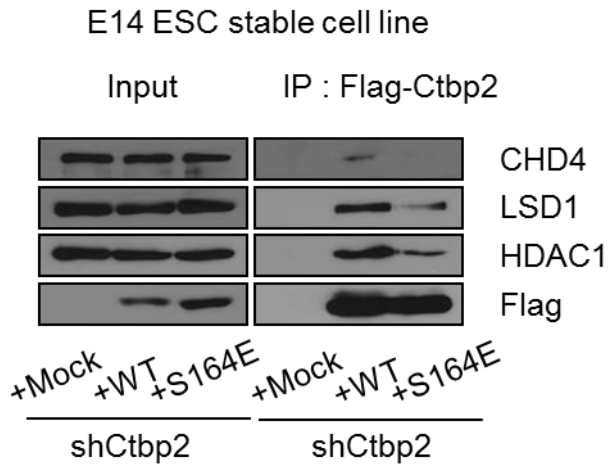

Figure 3-3-10. CTBP2 S164E mutant dissociates with NuRD complex in mESCs.

ESCs which stably overexpressed wildtype or S164R mutant CTBP2 were immunoprecipitated with anti-Flag-bead. Wildtype CTBP2 binds to endogenous CHD4, LSD1 and HDAC1. Compare with wildtype, S164E mutant Ctbp2 exhibits reduced binding ability.

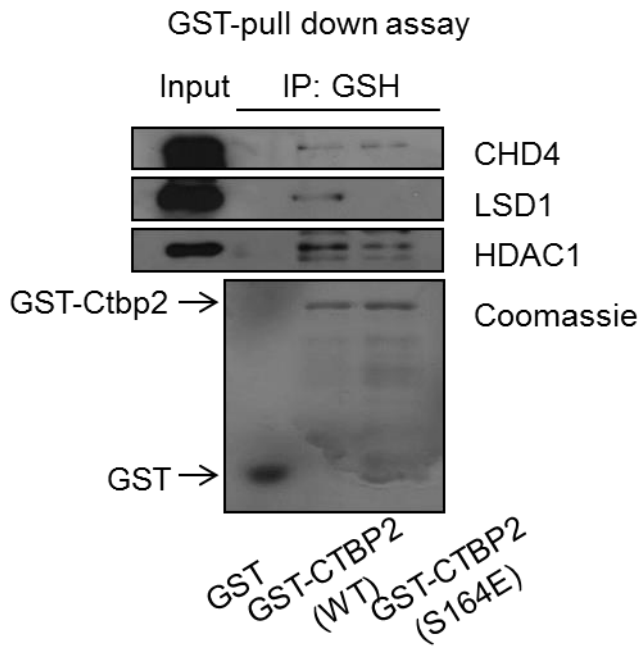

Figure 3-3-11. CTBP2 S164E mutant dissociated with NuRD complex.

Wildtype or S164E mutant GST-CTBP2 incubate with E14 cell lysates. Lysate precipitated with glutathione sepharose bead. Wildtype CTBP2 binds to endogenous CHD4, LSD1 and HDAC1. Compare with wildtype, S164E mutant Ctbp2 exhibits reduced binding ability.

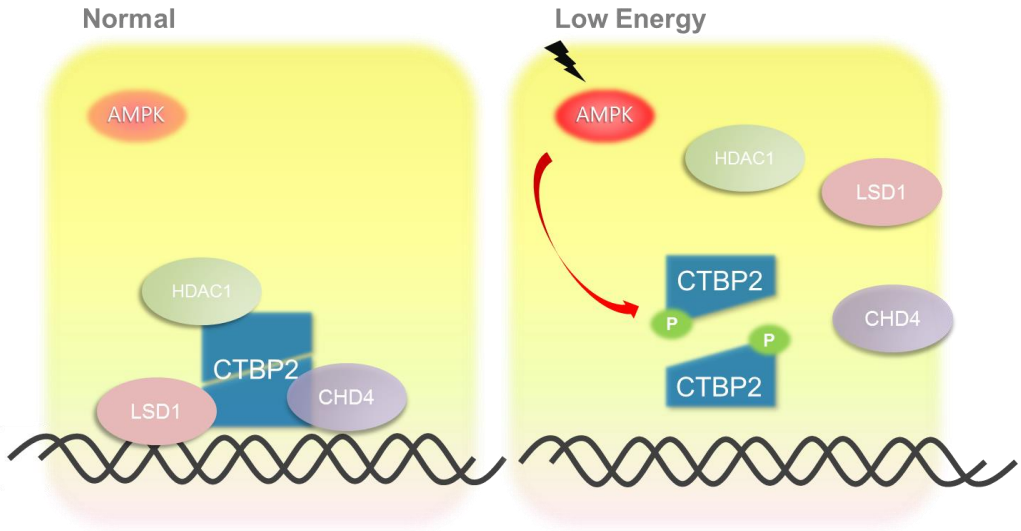

Figure 3-3-12. A Schematic model.

In energy deprivation, AMPK phosphorylates Ctbp2 at S164. Phosphorylated Ctbp2 dissociates with NuRD complex and leads to incomplete exit from pluripotency during differentiation.

IV. DISCUSSION

Energy balance and proper metabolic status are crucial for cell viability. Stem cells have a distinct feature compared with somatic cells in metabolic status. In contrast to somatic cells, ESCs rely on glycolysis than oxidative phosphorylation for energy supply (Leese HJ et al., 1984). In developmental process, ESCs exhibit distinct metabolic phenotype as per state of differentiation (Folmes C.D, 2012). Furthermore, in reprogramming process, somatic cells undergo metabolic shift to depending on glycolytic metabolism (Takahasi K et al., 2006; Folmes C.D et al., 2011a,b). Thus, nutrients and metabolic status are regarded as having a critical role in stemness and lineage specification although it is not well understood.

AMPK serves as energy gauge of cell. AMPK recognize deprivation of cellular energy (Carling D et al., 1989; Woods A et al., 2005; Rafaeloff-Phail et al., 2004). In energy stress state such as low-glucose, heat-shock, and hypoxia, AMPK is activated and induces catabolic pathway to generate ATP and repress anabolic pathway to inhibit ATP consumption (Corton JM et al., 1994; Minokoshi et al., 2001; Merrill G.F et al., 1997, Muoio DM et al., 1999). In stem cells, previous study demonstrated that activation of AMPK up-regulates pluripotency related genes and exhibits delayed differentiation (Adamo L et al., 2009; Shi X et al., 2013).

Ctbp regulates various genes expression which are related in pluripotency and differentiation by interacting with epigenetic modifier such as PRC and NuRD complex (Schaeper U et al., 1995; Sundqvist A et al., 1998; Koipally J et al., 2000; Sewalt RG et at., 1999). Moreover, Ctbp2 is a component of Oct4 interactome (Esch et al., 2012). And Ctbp2 deficient-ESCs

sustain pluripotency during differentiation process (Tarleton HP et al., 2010).

We recently revealed that AMPK phosphorylates Ctbp1 and down-regulates its activity and Ctbp2 is essential for exit from pluripotency (Kim et al., 2013; Kim et al., 2015). Therefore, we postulate that in energy deprivation, AMPK retards exit from pluripotency during differentiation by phosphorylating Ctbp2. First, to determine molecular function of AMPK in stem cells, we activate AMPK by 2DG or metformin. As a results, AMPK activated-ESCs exhibit retarded exit from pluripotency. These results reveal that stem cell differentiation ability is regulated by energy-dependent manner. Next, we sought to determine mechanism of stem cells regulated by cellular energy. Since AMPK phosphorylates Ctbp1 and that site is conserved in Ctbp2, we investigated AMPK modulates Ctbp2. As expected, AMPK phosphorylates Ctbp2. Furthermore, Phosphorylated Ctbp2 loses ability to dimerize and dissociates with NuRD complex.

Collectively, in this study, we reveal that in energy deprivation, stem cells sense metabolic states and retard exit from pluripotency through AMPK/Ctbp2. Based on this results, we suggest that stem cells block inappropriate differentiate by energy sensing pathway such as AMPK. Extensive study evidenced that not only AMPK but also other energy sensing-pathways such as AKT, mTOR and PAK are regulates pluripotency. Moreover, because Ctbp acts as homo/hetero dimer which is mediated by NAD+ or NADH and Ctbp has a higher affinity for NADH than NAD+, Ctbp functions as redox sensor. It suggests that cellular energy level regulates Ctbp and various energy sensing signal proteins and modulates stem cell differentiation.

V. REFERENCES

Adamo,L.,Naveiras,O.,Wenzel,P.L.,McKinney-Freeman,S.,Mack,P.J.,Gracia-Sancho,J.,Suchy-Dicey,A.,Yoshimoto,M.,Lensch,M.W.,Yoder,M.C., et al. (2009). Biomechanical forces promote embryonic haematopoiesis. Nature 459, 1131-1135.

Bandow,K., Kusuyama,J., Kakimoto,K., Ohnishi,T., and Matsuguchi,T.(2015). AMP-activated protein kinase(AMPK) activity negatively regulates chondrogenic differentiation. Bone 74, 125-133.

Carling, D., Clarke, P.R., Zammit, V.A., and Hardie, D.G. (1989).
Purification and characterization of the AMP-activated protein kinase. Copurification of acetyl-CoA carboxylase kinase and 3-hydroxy-3-methylglutaryl-CoA reductase kinase activities. European journal of biochemistry / FEBS 186, 129-136.

Conaghan, J., Handyside, A.H., Winston, R.M., and Leese, H.J. (1993). Effects of pyruvate and glucose on the development of human preimplantation embryos in vitro. Journal of reproduction and fertility 99, 87-95.

Corton, J.M., Gillespie, J.G., and Hardie, D.G. (1994).
Role of the AMP-activated protein kinase in the cellular stress response. Current biology : CB 4, 315-324.

Corton, J.M., Gillespie, J.G., Hawley, S.A., and Hardie, D.G. (1995). 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells? European journal of biochemistry / FEBS 229, 558-565.

Dumollard, R., Ward, Z., Carroll, J., and Duchen, M.R. (2007). Regulation of redox metabolism in the mouse oocyte and embryo. Development 134, 455-465.

Esch, D., Vahokoski, J., Groves, M.R., Pogenberg, V., Cojocaru, V., Vom Bruch, H., Han, D., Drexler, H.C., Arauzo-Bravo, M.J., Ng, C.K., et al. (2013). A unique Oct4 interface is crucial for reprogramming to pluripotency. Nature cell biology 15, 295-301.

Faubert, B., Boily, G., Izreig, S., Griss, T., Samborska, B., Dong, Z., Dupuy, F., Chambers, C., Fuerth, B.J., Viollet, B., et al. (2013). AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. Cell metabolism 17, 113-124.

Folmes, C.D., Dzeja, P.P., Nelson, T.J., and Terzic, A. (2012). Metabolic plasticity in stem cell homeostasis and differentiation. Cell stem cell 11, 596-606.

Folmes, C.D., Nelson, T.J., Martinez-Fernandez, A., Arrell, D.K., Lindor, J.Z., Dzeja, P.P., Ikeda, Y., Perez-Terzic, C., and Terzic, A. (2011a). Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming. Cell metabolism 14, 264-271.

Folmes, C.D., Nelson, T.J., and Terzic, A. (2011b). Energy metabolism in nuclear reprogramming. Biomarkers in medicine 5, 715-729.

Grooteclaes, M.L., and Frisch, S.M. (2000). Evidence for a function of CtBP in epithelial gene regulation and anoikis. Oncogene 19, 3823-3828.

Hawley, S.A., Davison, M., Woods, A., Davies, S.P., Beri, R.K., Carling, D., and Hardie, D.G. (1996). Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. The Journal of biological chemistry 271, 27879-27887.

Hildebrand, J.D., and Soriano, P. (2002). Overlapping and Unique Roles for C-Terminal Binding Protein 1 (CtBP1) and CtBP2 during Mouse Development. Molecular and Cellular Biology 22, 5296-5307.

Jang, H., Kim, T.W., Yoon, S., Choi, S.Y., Kang, T.W., Kim, S.Y., Kwon, Y.W., Cho, E.J., and Youn, H.D. (2012). O-GlcNAc regulates pluripotency and

reprogramming by directly acting on core components of the pluripotency network. Cell stem cell 11, 62-74.

Kim, J.H., Choi, S.Y., Kang, B.H., Lee, S.M., Park, H.S., Kang, G.Y., Bang, J.Y., Cho, E.J., and Youn, H.D. (2013). AMP-activated protein kinase phosphorylates CtBP1 and down-regulates its activity. Biochemical and biophysical research communications 431, 8-13.

Kim, T.W., Kang, B.H., Jang, H., Kwak, S., Shin, J., Kim, H., Lee, S.E., Lee, S.M., Lee, J.H., Kim, J.H., et al. (2015). Ctbp2 Modulates NuRD-Mediated Deacetylation of H3K27 and Facilitates PRC2-Mediated H3K27me3 in Active Embryonic Stem Cell Genes During Exit from Pluripotency. Stem cells 33, 2442-2455.

Koipally, J., and Georgopoulos, K. (2000). Ikaros interactions with CtBP reveal a repression mechanism that is independent of Histone deacetylase activity. The Journal of biological chemistry 275, 19594-19602.

Leese, H.J., and Barton, A.M. (1984). Pyruvate and glucose uptake by mouse ova and preimplantation embryos. Journal of reproduction and fertility 72, 9-13.

Minokoshi, Y., Kim, Y.B., Peroni, O.D., Fryer, L.G., Muller, C., Carling, D., and Kahn, B.B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415, 339-343

Merrill, G.F., Kurth, E.J., Hardie, D.G., and Winder, W.W. (1997). AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. The American journal of physiology 273, E1107-1112.

Mochizuki, H., Ohnuki, Y., and Kurosawa, H. (2011). Effect of glucose concentration during embryoid body (EB) formation from mouse embryonic stem cells on EB growth and cell differentiation. Journal of bioscience and bioengineering 111, 92-97.

Muoio, D.M., Seefeld, K., Witters, L.A., and Coleman, R.A. (1999). AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. The Biochemical journal 338 (Pt 3), 783-791.

Oburoglu, L., Tardito, S., Fritz, V., de Barros, S.C., Merida, P., Craveiro, M., Mamede, J., Cretenet, G., Mongellaz, C., An, X., et al. (2014). Glucose and glutamine metabolism regulate human hematopoietic stem cell lineage specification. Cell stem cell 15, 169-184.

Quinn, P. (1995). Enhanced results in mouse and human embryo culture using a modified human tubal fluid medium lacking glucose and phosphate. Journal of assisted reproduction and genetics 12, 97-105.

Rafaeloff-Phail, R., Ding, L., Conner, L., Yeh, W.K., McClure, D., Guo, H., Emerson, K., and Brooks, H. (2004). Biochemical regulation of mammalian AMP-activated protein kinase activity by NAD and NADH. The Journal of biological chemistry 279, 52934-52939.

Reynolds, N., Latos, P., Hynes-Allen, A., Loos, R., Leaford, D., O'Shaughnessy, A., Mosaku, O., Signolet, J., Brennecke, P., Kalkan, T., et al. (2012). NuRD suppresses pluripotency gene expression to promote transcriptional heterogeneity and lineage commitment. Cell stem cell 10, 583-594.

Schaeper, U., Boyd, J.M., Verma, S., Uhlmann, E., Subramanian, T., and Chinnadurai, G. (1995). Molecular cloning and characterization of a cellular phosphoprotein that interacts with a conserved C-terminal domain of adenovirus E1A involved in negative modulation of oncogenic transformation. Proceedings of the National Academy of Sciences of the United States of America 92, 10467-10471.

Sewalt, R.G., Gunster, M.J., van der Vlag, J., Satijn, D.P., and Otte, A.P. (1999). C-Terminal binding protein is a transcriptional repressor that interacts

with a specific class of vertebrate Polycomb proteins. Mol Cell Biol 19, 777-787.

Smith, J., Ladi, E., Mayer-Proschel, M., and Noble, M. (2000). Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. Proceedings of the National Academy of Sciences of the United States of America 97, 10032-10037.

Sundqvist, A., Sollerbrant, K., and Svensson, C. (1998). The carboxy-terminal region of adenovirus E1A activates transcription through targeting of a C-terminal binding protein-Histone deacetylase complex. FEBS letters 429, 183-188.

Suzuki, A., Okamoto, S., Lee, S., Saito, K., Shiuchi, T., and Minokoshi, Y. (2007). Leptin stimulates fatty acid oxidation and peroxisome proliferator-activated receptor alpha gene expression in mouse C2C12 myoblasts by changing the subcellular localization of the alpha2 form of AMP-activated protein kinase. Mol Cell Biol 27, 4317-4327.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676.

Tarleton, H.P., and Lemischka, I.R. (2010). Delayed differentiation in embryonic stem cells and mesodermal progenitors in the absence of CtBP2. Mechanisms of development 127, 107-119.

Thio, S.S., Bonventre, J.V., and Hsu, S.I. (2004). The CtBP2 co-repressor is regulated by NADH-dependent dimerization and possesses a novel N-terminal repression domain. Nucleic acids research 32, 1836-1847.

Vazquez-Martin,A. ,Vellon,L. ,Quiros,P.M., Cufi,S. ,Ruizde Galarreta,E., Oliveras-Ferraros,C., Martin,A.G., Martin-Castillo,B., Lopez-Otin,C., and Menendez,J.A. (2012). Activation of AMP-activated protein kinase (AMPK) provides a metabolic barrier to reprogramming somatic cells into stem cells. Cell cycle (Georgetown,Tex) 11, 974-989.

Wang, Q., Liang, B., Shirwany, N.A., and Zou, M.H. (2011). 2-Deoxy-D-glucose treatment of endothelial cells induces autophagy by reactive oxygen species-mediated activation of the AMP-activated protein kinase. PloS one 6, e17234.

Wiley, C., and Campisi, J. (2014). NAD+ controls neural stem cell fate in the aging brain. The EMBO journal 33, 1289-1291.

Williamson, D.L., Butler, D.C., and Alway, S.E. (2009). AMPK inhibits myoblast differentiation through a PGC-1alpha-dependent mechanism.

American journal of physiology Endocrinology and metabolism 297, E304-314.

Winder, W.W., and Hardie, D.G. (1999). AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. The American journal of physiology 277, E1-10.

Woods, A., Dickerson, K., Heath, R., Hong, S.P., Momcilovic, M., Johnstone, S.R., Carlson, M., and Carling, D. (2005). Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell metabolism 2, 21-33.

Woods, A., Johnstone, S.R., Dickerson, K., Leiper, F.C., Fryer, L.G.D., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M., and Carling, D. (2003). LKB1 Is the Upstream Kinase in the AMP-Activated Protein Kinase Cascade. Current Biology 13, 2004-2008.

Shi, X., Wu, Y., Ai, Z., Liu, X., Yang, L., Du, J., Shao, J., Guo, Z., and Zhang, Y. (2013). AICAR sustains J1 mouse embryonic stem cell self-renewal and pluripotency by regulating transcription factor and epigenetic modulator expression. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 32, 459-475

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., et al. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. The Journal of clinical investigation 108, 1167-1174.

Ⅵ. ABSTRACT IN KOREAN

국문 초록

줄기세포는 분화 과정에 따라 특이적인 metabolic phenotype 을 보이며, 이러한 특이적인 특성은 줄기세포가 'stemness' 한 성질을 유지하는데 중요하게 작용하는 것으로 생각된다. AMPK(AMP-activated protein kinase)는 세포의 에너지 상태를 인지하여 에너지 고갈 상태에서 활성화 되는 에너지 인지 인산화 효소이다. 하지만, 줄기세포에서의 그 기능은 명확히 알려진 바 없다. Ctbp(C-terminal binding protein)는 co-repressor 로써, 다양한 유전자들의 전사활성을 조절하는 것으로 알려져 있으며, 최근 연구결과는 Ctbp2 가 줄기세포 분화과정에서 중요한 역할을 한다는 사실을 밝힌 바 있다. 더욱이, AMPK 가 Ctbp1 을 인산화 함으로써 그 기능을 저해함이 알려져 있다. 따라서 본 연구에서는 줄기세포에서 에너지 상태에 따라 AMPK 가 Ctbp2 를 인산화 함으로써 pluripotency 탈출을 조절 할 수 있음을 가정하고 이를 알아보고자 하였다. 우선, Ctbp1 의 S158 AMPK 인산화 자리는 Ctbp2 의 164 번에 잘 보존되어있었고, AMPK 를 통해 인산화 됨을 확인하였다. AMPK 에 의해 S164 가 인산화 된 Ctbp2 는 dimer 를 형성 하지 못하고 NuRD complex 와 결합이 저해되며, 그로 인하여 줄기세포의 분화가 지연되는 현상을 확인하였다. 결론적으로 AMPK 가 줄기세포의 에너지 상태를 인지하고, Ctbp2 를 통하여

분화를 조절 할 수 있음을 밝혔다. 이것은 줄기세포가 불안정한 에너지 상태에서는 분화를 저지 할 수 있는 기작이 존재함을 암시한다.

주요어 : 줄기세포, 에너지, AMPK (AMP-activated protein kinase), Ctbp (C-terminal binding protein)

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