Master of Science

Transcription factor-EB supports pancreatic cancer growth through regulation of glutamine metabolism.

췌장암 글루타민 대사에서
Transcription factor-EB의 역할 규명

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Transcription factor-EB supports pancreatic cancer growth through regulation of glutamine metabolism

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Transcription factor-EB supports pancreatic cancer growth through regulation of glutamine metabolism

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ABSTRACTS

Transcription factor EB (TFEB) is a master regulator of lysosomal function and autophagy. Autophagy is critical to maintain energy homeostasis in pancreatic cancer cells. However, the precise roles of TFEB in pancreatic cancer growth remain unclear. Here I show that TFEB regulates glutamine metabolism, which is indispensable for pancreatic cancer growth. Pancreatic ductal adenocarcinoma (PDAC) cells require glutamine metabolism to maintain cell survival and growth. In my study, the knockdown of TFEB significantly impairs PDAC cell growth. Interestingly, TFEB knockdown has no significant effect on glucose metabolism, but markedly inhibits glutamine metabolism and results in a profound reduction of glutaminase (GLS) transcription, which converts glutamine into glutamate. In addition, I found that glutamate supplementation, but not glutamine, leads to a dramatic recovery of PDAC growth reduced by TFEB knockdown. I also observed that oncogenic c-Myc levels are decreased following TFEB knockdown. Taken together, my data demonstrate that TFEB supports the PDAC cell growth by regulating glutaminase through oncogene c-Myc.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is most common form of pancreatic cancer, and one of the most fetal malignancies in humans. It is fourth leading cause of cancer death worldwide with a 5-year survival rate of less than 5%.[1, 2] In the last decades, diagnostic methods to detect pancreatic cancer have been improved. However, the death rate of pancreatic cancer is not advanced in the past few decades. The prognosis of pancreatic cancer is also still worse because of the lack of specific symptoms in the early stage and the difficulty of medical treatment.[3, 4] Pancreatic cancers are tremendously resistant to cytotoxic chemotherapies and radiotherapy, and the location of the tumor also causes surgical difficulties. Therefore, it is essential to develop new-agents to treat the pancreatic cancer.

Cancer metabolism is a new field of active research worldwide, included in the last cancer hallmark and making it the novel study of cancer diagnostics and therapeutic drugs to be published in 2011.[5] Unlike normal cells, cancer cells increase the uptake of nutrients for proliferation. To support survival and biosynthesis, glucose and glutamine is utilized as carbon sources to generate ATP and maintain cellular redox homeostasis. Glucose mainly enters pentose phosphate pathway (PPP) through glycolysis to generate building blocks for proliferation.[5, 6] This pathway is called as the Warburg effect, which is called aerobic glycolysis and indicated that the glycolysis and lactate production is increased in the cancer cells regardless of oxygen existence.[6, 7] However, the production of ATP that can be obtained with glycolysis is considerably inefficient compared to using TCA cycle.[8] Therefore, many cancer cells utilize glutamine for generating TCA cycle intermediates.

Recently, many studies have reported that many cancer cells highly require glutamine for growth and survival.[9] This is known as glutaminolysis. Glutamine is used for carbon source in the TCA cycle and is the primary nitrogen donor for the synthesis of nucleotides and nonessential amino acids.[10] Glutamine enters the TCA cycle by converting into glutamate by glutaminase. Glutamine can produce large amounts of ATP, NADH, NADPH, and NEAA, etc., which plays an important role in many cancer cells that exhibit the Warburg effect.[10, 11] Because of these properties of glutamine, glutaminolysis is an important part of cancer metabolism.

Transcription factor-EB (TFEB) is a member of the microphthalmia family of basic helix-loop-

helix – leucine-zipper (bHLH-Zip) transcription factors (MiT family) and is well known as a master regulator of lysosomal function and autophagy.[12, 13, 14] TFEB regulates the expression by binding the promoter regions of lysosomal and autophagy related genes.[15, 16] TFEB also induces lysosomal exocytosis, and these properties of TFEB suggest that TFEB mediates the central cellular degradative pathways and intracellular clearance.[12, 17] The activity of TFEB is mainly regulated by phosphorylation.[15, 18] The phosphorylation of TFEB occurs by mTORC1 and ERK2 pathways. [19, 20, 21] When TFEB is phosphorylated, TFEB remains inactive in the cytosol.[15, 22] In nutrient deprivation conditions or stress conditions, TFEB can be activated and translocated in the nucleus. Then, active TFEB induces the upregulation of lysosomal or autophagy genes at the transcriptional levels to overcome this conditions.[23]

Autophagy is essential for PDAC growth in vitro and in vivo.[24, 25, 26] However, the function of TFEB in PDAC growth is not clear. In this study, I demonstrate that TFEB transcriptionally regulates GLS, which is essential for maintenance of glutamate levels.

MATERIALS AND METHODS

Cell culture and Reagents

All PDAC cell lines were obtained from the American Type Culture Collection and were regularly tested for mycoplasma contamination. All cells were maintained in a 5% CO2 atmosphere at 37℃ in DMEM(Thermo Scientific, Waltham, MA, USA). DMEM was mixed with 10% fetal bovine serum (FBS) and 100 U/ml penicillin, and 100 μg/mL streptomycin (Thermo Scientific, USA).

Plasmids

Primers were designed based on cDNA of PDAC cell lines and used in PCR to amplify c-Myc and TFEB. First, TFEB was amplified by PCR with the following two primers (TFEB-F ; CGC GAA TTC GCC ACC ATG GCG TCA CGC ATA GGG, TFEB-B ; CGC GGA TCC TCA CAG CAC ATC GCC CTC), digested, and cloned into EcoR I - BamH I sites (underlined) of pCDH vector. The cDNAs of human c-Myc was obtained by PCR with the following primers (c-Myc F ; CGG AAT TCA TGC CCC TCA ACG TTA GCT TCAC, c-Myc B ; CCG CTC GAG TTA CGC ACA AGA GTT CCG TAG CTG), cut by EcoR I and Xho I, and ligated with pENTR4-Dual Selection vector. The c-Myc DNA within pENTR4-Dual vector was transferred into pLenti6.2_v5_DEST vector by Gateway system.

Lentiviral-mediated shRNA

The RNAi Consortium clone IDs for the RNAs used in this study are as follows:
TRCN0000013112 (shTFEB-1; TGATCCACTTCTGTCCACCAT) and TRCN0000013109 (shTFEB-2; CGATGTCCTTGGCTACATCAA).

Quantitative real-time PCR

RNA was isolated with TRIzol (QIAGEN) and cDNA was synthesized using 2μg of RNA with MMLV HP reverse transcriptase (Epicentre, Madison, WI, USA). Quantitative real-time PCR was performed with SYBR Green dye using an ArizMx Real-Time PCR system. The relative amount of cDNA was calculated by the comparative Ct method using the 18S ribosomal RNA sequences as a

control. The primer sequences were designed as follows; 18S (Forward ; GTA ACC CGT TGA ACC CCA TT, Reverse ; CCA TCC AAT CGG TAG TAG CG), GLS (Forward; GCT GTG CTC CAT TGA AGT GA, Reverse; GCA AAC TGC CCT GAG AAG TC), Myc (Forward; TCC CTC CAC TCG GAA GGA C, Reverse; CTG GTG CAT TTT CGG TTG TTG)

Metabolomics

Cell were grown to about 60% confluence in growth media (DMEM, 10%FBS, 5%Penicillin) on 10cm dishes in biological triplicate. After 24hr, the cell then were harvested using 1.4mL of cold Methanol/H_2O (80/20, v/v) after sequential wash with PBS and H_2O and lysed by vigorous vortexing and 100mL of 5uM of internal standard was added. Metabolites were extracted from aqueous phase by liquid-liquid extraction after adding chloroform. The aqueous phase was dried using vacuum centrifuge, and the sample was reconstituted with 50ul of 50% methanol prior to LC-MS/MS analysis. LC/MS/MS system was equipped with Agilent 1290 HPLC (Agilent) and Qtrap 5500(ABSciex), and reverse phase column (Synergi fusion RP50 x 2mm) was used. 3ul was injected into the LC-MS/MS system and ionized with turbo spray ionization source. Multiple reactions monitoring (MRM) was used in negative ion mode, and the extracted ion chromatogram (EIC) corresponding to the specific transition for each metabolite was used for quantitation. Area under the curve of each EIC was normalized to that of EIC of internal standard. Peak area ratio of each metabolite to internal standard was normalized using protein amount in a sample, and then was used for relative comparison.

Western blot

Cells were lysed in RIPA lysis buffer containing protein inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). The lysate concentration was analyzed with BCA assay (Thermo Scientific, Waltham, MA, USA). Equal amounts of lysates were mixed with H2O and Laemmli loading dye and boiled for 5 min. The lysates were loaded for SDS-PAGE and the proteins were transferred to PVDF membranes (EMD Millipore, Billerica, MA, USA). Membranes were blocked with 5% non-fat skim milk in Tris-buffered saline and 0.1% Tween 20(TBS-T), and incubated with primary antibody at 4℃ for overnight. After washing with TBS-T, membranes were exposed to the appropriate horseradish peroxidase-conjugated secondary antibody for 1hr at Room temperature. The protein-antibodies

complexes were visualized on LAS 2000 using an enhanced chemiluminescence (ECL) detection system (Thermo Scientific). Antibody to TFEB (A303-673A) was purchased from Bethyl Laboratories (Montgomery, TX, USA); Antibody to GLS (ab93434) was obtained from Abcam (Cambridge, MA, USA); Antibodies to c-Myc (sc-40) and actin (sc-47778) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

Cell proliferation assay

For Growth curve assay, cells were plated in 24-well plates at 2 x 10^3 cells per well in 0.5ml of media. On the indicated time points, cells were fixed in 10% buffered formalin and stained with 0.1% crystal violet (Sigma). Dye was extracted with 10% acetic acid and the relative proliferation was quantified by OD at 594nm.

Clonogenic assay

For Clonogenic assay, cells were plated in 6-well plates at 4 x 10^2 cells per well in 2ml of media. Media was not changed throughout the course of the experiment and cells were stained with 0.2% crystal violet (Sigma) after 10~12 days.

Measurement of OCR

Oxygen consumption rate (OCR) was measured with an XF24 extracellular flux analyzer (Seahorse Bioscience). Briefly, cells were plated in a 24-well Seahorse plate and cultured at 37℃ with 5% CO2, which was replaced the following day with unbuffered DMEM and incubated at 37℃ without CO2 for 1hr. Then, compounds were injected to the final concentration as oligomycin (2μM), FCCP (5μM), and Rotenone (2μM).

Intracellular ATP assay

Intracellular ATP concentrations were measured using an ATP Colorimetric/Fluorometric Assay Kit (Biovision Incorporated, Milpitas, CA, USA) according to the manufacturer's instructions. Briefly, cells were lysed in 100μl of ATP assay buffer; 50ul of supernatants were collected and added to a 96well plate. To each well, 50ul of ATP assay buffer containing ATP probe, ATP converter, and

developer were added. Absorbance was measured at 570nm.

Luciferase assay

One day before transfection, cells were plated 1~2 x 10^5 cells per well in a 12-well plate in 1ml of growth media so that will be 90~95% confluent at the time of transfection. Cells were transfected using Lipofectamine 2000 (Invitrogen) and serum free media with 600ng of c-Myc promoter and control plasmids and 1μg of β-gal DNA. Cells were incubated at 37℃ in a CO2 incubator for 4~ 6 hours. After incubation, the medium have to be replaced with complete medium. Then, cells were incubated at 37℃ in a CO2 incubator for 18~24 hours prior to testing for transgene expression. Cells were harvested and lysed using RIPA lysis buffer containing protein inhibitor cocktail. Luciferase activities were measured using Luciferase Assay System (Promega).

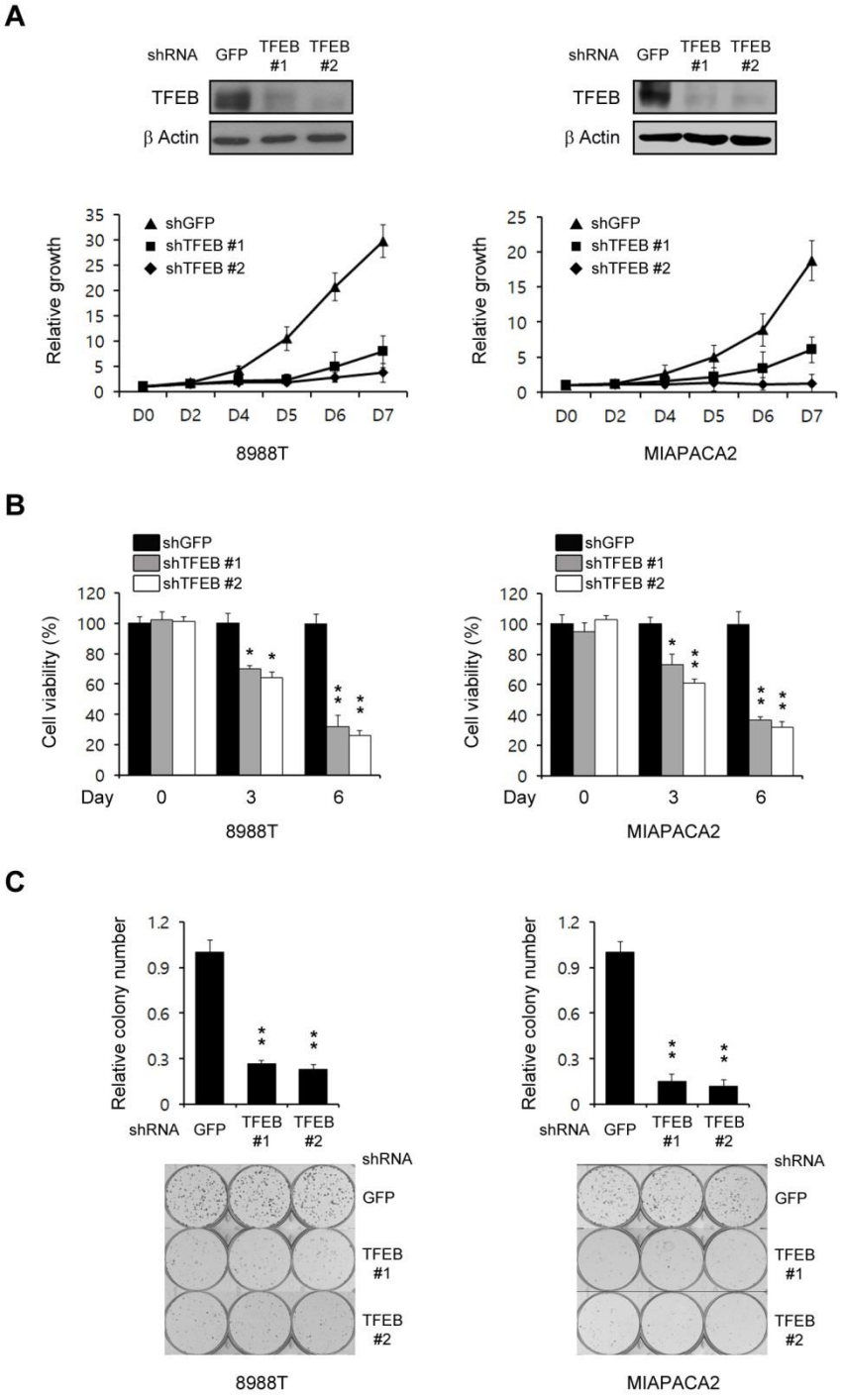
Statistics

Data are presented as mean ± standard deviation. All comparisons were analyzed using unpaired Student's t-test.

RESULTS

1. TFEB is essential for PDAC growth.

TFEB is well known as a critical regulator of autophagy.[12, 13] It has been shown that TFEB-regulated autophagy is essential for some cancer growth.[14, 24] However, the roles of TFEB in cancer metabolism is not yet clear. To explore whether TFEB relates to cancer metabolism, I first investigated the effect of Knockdown of TFEB on growth of pancreatic cancer. I used two PDAC cell lines, 8988T and Miapaca2, harboring KRas mutation. I constructed TFEB knockdown 8988T and Miapaca2 cells by Lenti-virus infection and confirmed TFEB knockdown by western blot. Knockdown of TFEB dramatically inhibited cell proliferation in 8988T and Miapaca2 cells (Fig. 1A). To confirm these results, I examined MTT and Clonogenic assay. As shown in Fig. 1B and C, the results are consistent with my previous finding that knockdown of TFEB significantly decreased cell proliferation. Thus, these results suggest that TFEB is important for pancreatic cancer cell proliferation.


A
shRNA GFP TFEB #1 TFEB #2
TFEB
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shRNA GFP TFEB #1 TFEB #2
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Relative growth
shGFP
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D0 D2 D4 D5 D6 D7
8988T
MIAPACA2
B
Cell viability (%)
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8988T
MIAPACA2
C
Relative colony number
shRNA GFP TFEB #1 TFEB #2
8988T
MIAPACA2

Figure 1. Effect of TFEB knockdown on PDAC growth. **A)** Cell growth in PDAC cell lines was performed using Growth curve assay. Data was decreased depending on TFEB knockdown. **B)** Cell viability was measured by MTT assay. It was reduced under knockdown of TFEB. **C)** Clonogenic assay was performed with TFEB knockdown in PDAC cell lines. Data shown cell colony number was decreased under knockdown of TFEB. Error bars represent the standard deviations (s.d.) of triplicate wells from a representative experiment. *, $p < 0.05$; **, $p < 0.01$.

2. TFEB influences the TCA cycle, but not glycolysis.

To examine the functional role of TFEB in PDAC metabolism, I first attempted to measure glycolysis and TCA cycle metabolites. I observed that glycolysis metabolites are not influenced by knockdown of TFEB (Fig. 2A). However, TCA cycle metabolites are significantly decreased upon TFEB knockdown (Fig. 2B). These results suggest that TFEB is related to TCA cycle, but not glycolysis. To confirm the effect of TFEB knockdown on TCA cycle, I examined the oxygen consumption rate and ATP level. As shown in Fig. 2C, oxygen consumption rate was decreased upon knockdown of TFEB. ATP levels were also significantly decreased by TFEB reduction (Fig. 2D). Taken together, these data demonstrate that TFEB is critical for maintenance of proper mitochondrial functions.

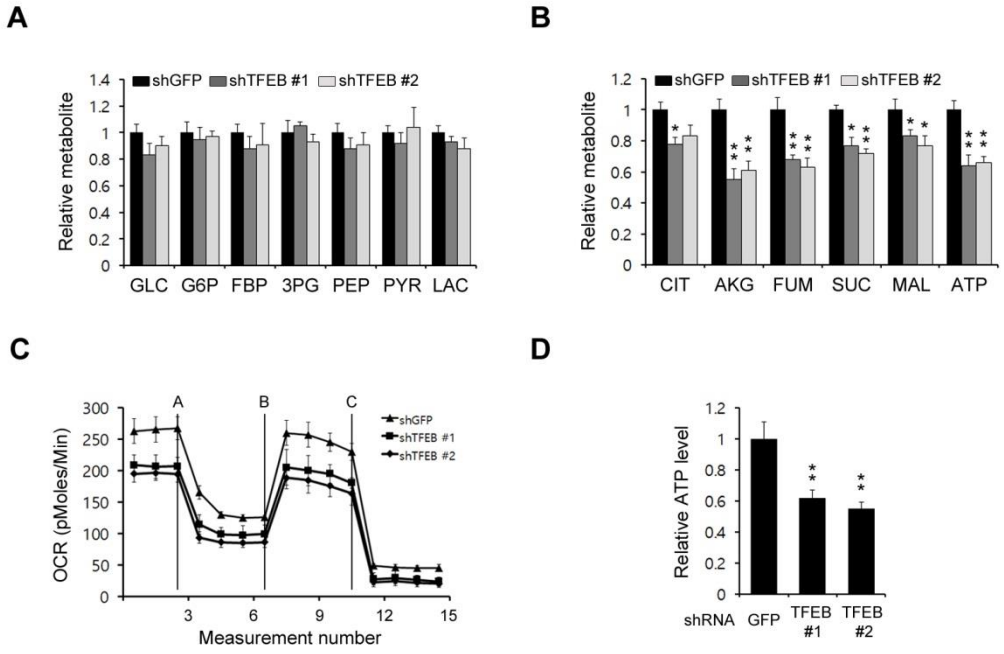

Figure 2. PDAC requires TFEB for maintaining proper mitochondrial functions. **A-B)** Glycolysis and TCA cycle metabolite pools were analyzed with LC-MS/MS in TFEB knockdown PDAC cells. TCA cycle metabolite pools were decreased, but glycolysis was maintained. **C)** Oxygen consumption rate (OCR) was measured with oligomycin (A; 2µM), FCCP (B; 5µM), and Rotenone (C; 2µM). Data presents OCR of TFEB knockdown cells was decreased compared to GFP control cells. **D)** ATP levels of TFEB knockdown cells was decreased compared to GFP control cells. Error bars represent the s.d. of triplicate wells from a representative experiment. *, $p < 0.05$; **, $p < 0.01$.

3. TFEB supports PDAC growth through regulation of glutaminolysis.

In normal cells, glucose is utilized to fuel the TCA cycle, whereas many cancer cells utilize glutamine as a source of carbon fuel for the TCA cycle. Pancreatic cancer cells also use glutamine as a carbon source for fueling TCA cycle.[27] To explore whether TFEB affects glutaminolysis, I measured the intracellular glutamine and glutamate levels in response to TFEB knockdown. The reduction of TFEB had no effect on glutamine (Gln) level, whereas it had significantly effect on glutamate (Glu) level (Fig. 3A). To validate that glutamate is essential for cell proliferation in TFEB knockdown cells, I performed to see if the reduced cell proliferation from TFEB knockdown can be rescued by glutamine or glutamate supplementation. As shown in Fig. 3B, Glutamine supplementation does not lead to a significant recovery of cell proliferation. However, glutamate supplementation significantly led to a recovery of cell proliferation. These results are suggesting that TFEB regulates intracellular glutamate levels.

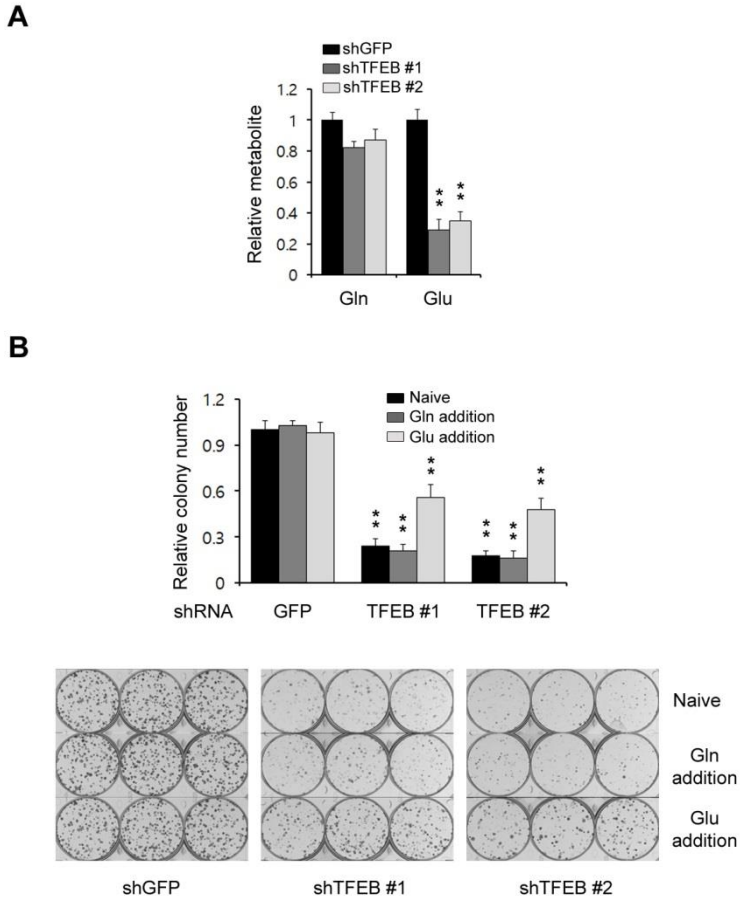

Figure 3. TFEB is critical for the maintenance of glutamate levels. **A)** Glutamine (Gln) and glutamate (Glu) metabolite was measured with LC-MS/MS in TFEB knockdown PDAC cells. Glutamine was not changed, but Glutamate was decreased. **B)** Cells was infected with pLKO-TFEB knockdown virus and treated with glutamine (4mM) and glutamate (4mM) and measured cell colony number by Clonogenic assay. Error bars represent the s.d. of triplicate wells from a representative experiment. **, $p < 0.01$.

4. TFEB regulates the expression of glutaminase through c-Myc.

Glutamine is converted into glutamate through Glutaminase (GLS) and glutamate is converted into α-ketoglutarate via glutamine dehydrogenase (GLUD1) or other transaminases (Fig. 4A). As intracellular glutamate levels are regulated by glutaminase (GLS), I assessed GLS levels following knockdown of TFEB. As shown in Fig. 4B, GLS protein and mRNA levels were significantly decreased. It has been reported that GLS is regulated by oncogene c-Myc.[28, 29] Therefore, to investigate whether c-Myc is involved in TFEB-mediated GLS regulation, I examined to confirm c-Myc levels in TFEB knockdown cells. c-Myc was remarkably reduced as TFEB was decreased (Fig. 4C). To determine whether TFEB actually regulates GLS through c-Myc, I overexpressed TFEB and c-Myc levels. When TFEB expression was up-regulated, c-Myc and GLS expression were also increased (Fig. 4D). I performed co-infection using TFEB knockdown virus and c-Myc over-expression virus. As shown in Fig. 4E, GLS was decreased according as TFEB is reduced. However, when c-Myc expression is increased in TFEB knockdown cells, GLS levels are recued. Taken together, these results suggested that TFEB regulates GLS levels through c-Myc.

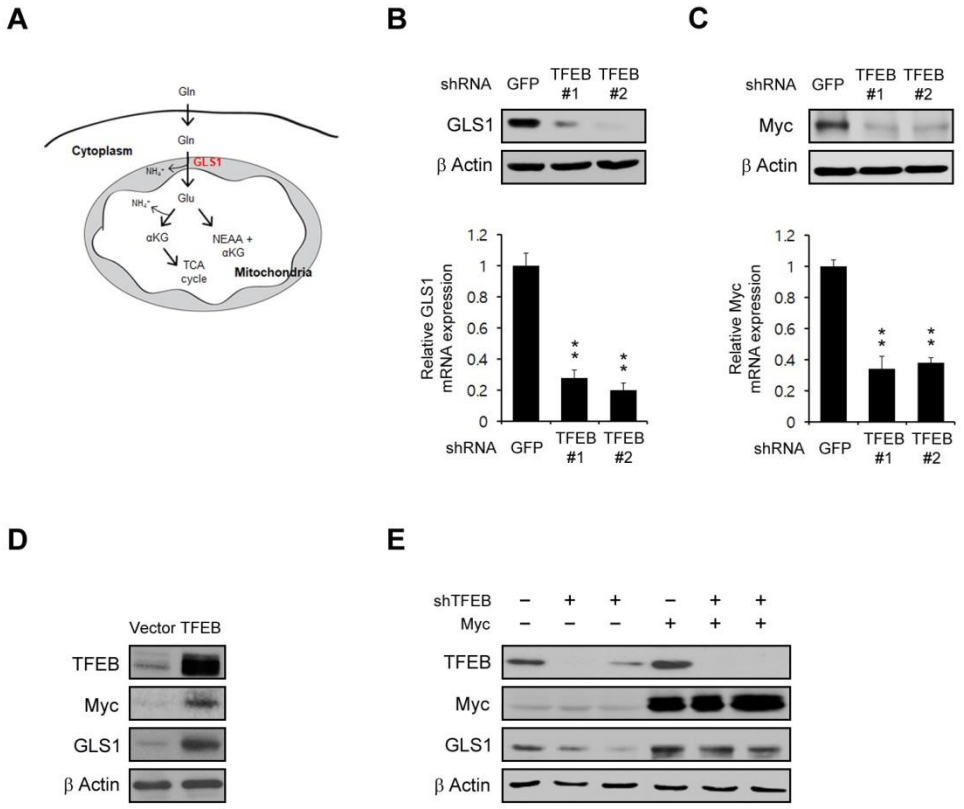

Figure 4. c-Myc is required for TFEB-mediated GLS regulation. **A)** Glutamine enters in mitochondria and converted into glutamate by Glutaminase 1 (GLS1; mitochondrial glutaminase). Glutamate is converted into α-ketoglutarate and making ammonium or non-essential amino acids (NEAA). α-ketoglutarate is entering the TCA cycle and used for generating ATP, NAD(P)H, etc. **B)** Protein levels of GLS were assayed by Western blot and mRNA expression of GLS was measured by Quantitative real time PCR analysis. Protein levels and mRNA expression of GLS was decreased in TFEB knockdown PDAC cells. **C)** Protein levels and mRNA expression of c-Myc was also decreased depending on TFEB knockdown. **D)** TFEB overexpression using pCDH-TFEB plasmids was induced the increase of c-Myc and GLS protein levels. **E)** Data was using pLKO-TFEB and pLenti6.2_v5_DEST_c-Myc plasmids. Error bars represent the s.d. of triplicate wells from a representative experiment. **, $p < 0.01$.

5. TFEB regulates c-Myc promoter activity.

My data revealed that TFEB regulated GLS levels through oncogene c-Myc. Thus, I next investigated how TFEB regulates the expression of c-Myc. Cells were transfected by c-Myc promoter plasmids with luciferase in TFEB knockdown cells. As shown in Fig. 5A, when TFEB was reduced, c-Myc promoter activity was also significantly decreased. This result indicates that TFEB regulated the transcription of c-Myc. To confirm that GLS is regulated by TFEB via c-Myc, I investigated luciferase assay with GLS promoter plasmids. When TFEB was decreased, GLS promoter activity was also reduced. However, the overexpression of c-Myc rescued GLS transcription levels under knockdown of TFEB (Fig. 5B). These data was indicated that TFEB regulated the expression of GLS through the controls of transcription of c-Myc.

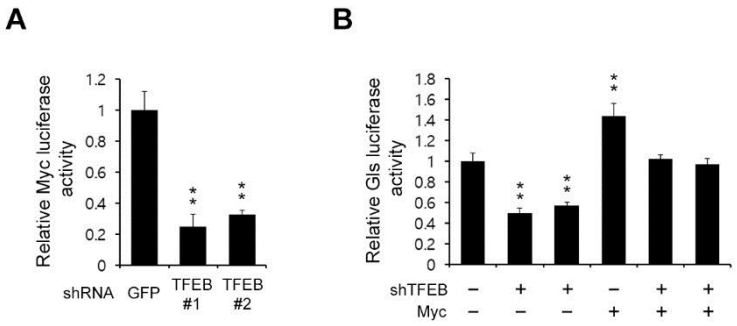

Figure 5. c-Myc promoter activity is controlled by TFEB. **A)** Luciferase assay was performed with pGL3-Luciferase and pGL3-c-Myc promoter plasmids. **B)** Luciferase assay was executed with GLS promoter in Co-infection PDAC cells of TFEB knockdown virus and c-Myc overexpression virus. **, $p < 0.01$.

DISCUSSION

TFEB is well known as a key regulator of lysosomal function and autophagy.[12, 13] TFEB is important for the cell growth of PDAC by regulating autophagy [14, 24, 25]. However, the roles of TFEB in PDAC growth are not clear. Thus, I explore to determine the mechanism by which TFEB participates in PDAC growth. I showed that PDAC growth was affected by the reduction of TFEB expression. In my study, I found that the reduction of TFEB levels decreased TCA cycle intermediates, but not Glycolysis. The decrease in TFEB also reduced the oxygen consumption rate (OCR) and ATP levels, and this data confirmed that the expression of TFEB is important for maintaining proper mitochondrial functions. The influx of carbon source into TCA cycle involves glucose uptake via glycolysis and glutamate uptake via glutaminolysis. Previous data showed that expression of TFEB is not associated with glycolysis, so intracellular concentrations of glutamine and glutamate have been identified. When the expression of TFEB was decreased, the concentration of glutamate was significantly decreased, which was confirmed by the rescue results of cell proliferation when treated with glutamate. In other words, these data indicate that TFEB reduction affects glutaminase, which mediates the conversion of glutamine into glutamate.

Glutaminase is a key enzyme of glutaminolysis and converts glutamine into glutamate; and previous studies have suggested that glutaminase is regulated by oncogene c-Myc.[28,29] Therefore, I thought that oncogene c-Myc was involved in TFEB-mediated GLS regulation. In my study, I found that TFEB controlled the expression of glutaminase by regulating c-Myc levels through western blot and promoter activity under TFEB knockdown. These results described that TFEB regulates the transcription levels of oncogene c-Myc and influences the expression of glutaminase.

In further study, I will investigate what region of c-Myc promoter is essential for the regulation by TFEB. Currently, I have prepared partially deleted c-Myc promoters and will use these for luciferase assay to explore what part of c-Myc promoter is associated with TFEB-mediated c-Myc expression. Lastly, these findings may have implications for future therapeutic approaches as inhibition of TFEB-mediated glutamine reprogramming in PDAC.

CONCLUSION

PDAC is the most common form of malignancy in the pancreas and has a re-programmed metabolism pathway. PDAC utilizes glucose via glycolysis and pentose phosphate pathway for continuous proliferation. Then, glutamine is essential for the TCA cycle of PDAC to maintain cellular energy homeostasis. Autophagy is the essential pathway that removes itself unnecessary or broken organelles and macromolecules. Transcription factor-EB (TFEB) is a master regulator of lysosomal and autophagy. In previous study, autophagy is required for the growth of pancreatic cancer and PDAC glutamine metabolism. However, the role of TFEB in pancreatic cancer remains unclear.

In this study, I found that TFEB supports the growth of PDAC. My data described that these effect of TFEB is related to TCA cycle, not glycolysis. Glutamine is a major carbon source of TCA cycle of PDAC; thus, when I measured the intracellular glutamine and glutamate concentration, I can observe that TFEB knockdown induces the reduction of Glutaminase (GLS) levels. In addition, c-Myc, which is a well-known regulator of glutaminase, is adjusted by TFEB. Therefore, these observations indicated that TFEB supports the growth of PDAC by regulating glutamine metabolism.

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Abstract in Korea

Transcription factor EB(TFEB)는 이전 연구들을 통해서 Lysosome의 기능과 autophagy와 관련된 유전자들을 조절하는 master regulator로서 알려져 왔다. 이러한 TFEB는 autophagy를 조절하여 세포 증식에 중요한 에너지를 공급하는 역할을 한다. 하지만, TFEB가 췌장암 세포 성장에서 어느 역할을 하는지에 대한 것은 아직 명확하게 밝혀지지 않았다. 본 논문에서는 TFEB가 glutamine metabolism을 통해 pancreatic cancer cells의 성장에서 중요한 역할을 한다는 것에 대해 연구하였다.

Pancreatic ductal adenocarcinoma(PDAC)은 세포의 성장과 생존에 있어서 glutamine metabolism을 필요로 한다. TFEB를 knockdown 하였을 때 PDAC의 성장이 감소하는 것을 보아 TFEB가 PDAC의 성장에 있어서 중요한 역할을 하는 것을 알 수 있었으며, 그러한 TFEB의 영향은 glycolysis가 아닌 TCA cycle에 영향을 준다는 것을 알 수 있었다. TCA cycle에 주요 탄소공급원인 glutamine level을 확인하는 실험을 하였고, TFEB를 knockdown 하였을 때 세포 내 glutamine level은 거의 변화가 없었지만, glutamate level이 현저하게 감소하였다. 또한 TFEB knockdown 시에 glutamine metabolism에서 glutamine을 glutamate로 전환시켜주는 효소인 glutaminase(GLS)의 발현이 감소하는 것을 확인하였다. TFEB를 knockdown으로 인한 PDAC 성장의 억제는 glutamate를 처리하였을 때, 감소되었던 세포의 성장이 회복하였음을 알 수 있었다. 이는 TFEB가 glutamine metabolism의 주요 효소인 GLS에 영향을 준다는 것을 의미한다. GLS는 종양유전자인 c-Myc에 의해 조절받는다는 이전 연구를 바탕으로 TFEB가 c-Myc 전사에 영향을 주는지 알아보기 위해 luciferase assay 실험을 진행하였고, 그 결과 TFEB에 의해 c-Myc의 promoter 부분이 영향을 받아 c-Myc의 발현이 감소하였음을 알 수 있었다.

결과적으로 본 연구 결과는 autophagy의 주요 조절자인 TFEB가 autophagy가 아닌 종양유전자인 c-Myc을 통해 GLS를 조절함으로써 PDAC cells의 성장에 있어 중요한 역할을 하는 glutamine metabolism을 원활하게 유지하는데 있어 중추적인 역할을 한다는 것을 의미한다. 그러므로 이 결과들은 PDAC을 표적하는 치료제의 개

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