석 사 학 위 논 문

USP41 Enhances Epithelial-mesenchymal Transition of Breast Cancer Cells through Snail Stabilization

계 명 대 학 교 대 학 원

의 학 과

윤 지 윤

지도교수 권 택 규

2 0 2 2 년 8 월

USP41 Enhances Epithelial-mesenchymal Transition of Breast Cancer Cells through Snail Stabilization

지 도 교 수　　권 택 규

이 논문을 석사학위 논문으로 제출함

2022년 8월

계 명 대 학 교 대 학 원

의학과 면역학 전공

윤　　지　　윤

윤지윤의 석사학위 논문을 인준함

주 심 박 종 욱

부 심 권 택 규

부 심 김 신

계 명 대 학 교 대 학 원

2022년 8월

Acknowledgement

논문을 제출하는 동안 많은 지도와 조언을 해주신 권택규 교수님께 감사의 마음을 전합니다. 권택규 교수님께서는 화학 전공이었던 제에게 생물학적 연구에 접할 수 있는 기회를 제공해주셨습니다. 권택규 교수님께서는 항상 열정을 가지시고 연구에 대해 토론해 주시고 관심을 가져주셨습니다. 졸업한 후에도 감사함을 잊지 않고 그리워할 것입니다. 또한 저를 위해 아낌없는 조언을 해주시고 많은 시간을 함께 해 주신 박종욱 교수님, 김신 교수님께도 감사의 말씀을 전합니다.

연구실의 박사님과 학생 동료들에게도 감사의 마음을 전합니다. 그분들은 저에게 때로는 많은 연구 기술과 지식을 알려주시는 선생님이었고, 때로는 어려운 시기를 함께 이겨낸 전우 같은 존재였습니다. 덕분에 2년 동안 많은 감정과 추억을 나누며 보낼 수 있었습니다.

마지막으로 가족과 연인에게 사랑과 감사를 전합니다. 그들은 제가 졸업할 때까지 믿음과 격려로 저를 지지해 주었습니다. 제가 대부분의 시간을 학교에서 보내고 그들과 함께하지 못했음에도 불구하고 그들은 저를 이해해주고 가장 큰 힘이 되어 준 존재입니다.

2022년 8월

윤지윤

Table of Contents

List of Figures

1. Introduction

Epithelial-mesenchymal transition (EMT) is a biological process that epithelial cells change into mesenchymal cells, thereby getting the character which no polarity and loss of cell adhesion. In tumor, this process is activation and moves freely through the blood stream (1). The EMT is also related to drug resistance (2). Therefore, understanding of EMT is important for cancer therapy. EMT transcription factors (EMT-TF) can suppress the expression of epithelial genes including Lamin1, E-cadherin and Claudin, as well as activate mesenchymal genes such as Fibronectin and Vimentin (3). EMT-TF include Snail family proteins, Zinc finger e-box binding (Zeb) homeobox family proteins and Twist family proteins (3). The expression of Snail is modulated by various signal pathways at the transcriptional and post-translational levels (4,5). Transcriptional regulation of Snail expression is modulated by several signaling pathways such as NF-kB, Notch, Wnt, TGF-β and PI3K-Akt. Snail is also regulated by ubiquitin proteasome system (6). β-TrCP1 destabilizes Snail through GSK3β -mediated phosphorylation (7). Snail not only effects on EMT but also correlates with tolerance to 5-fluorouracil in breast cancer and cisplatin in ovarian cancer (8-10). Snail has an important role in cancer like regulation stemness and invasion (11).

Ubiquitination is modulated by E1, E2 and E3 enzymes and leads to proteasome-mediated degradation of proteins (12). This post-translational modification process is reversed by deubiquitination. Deubiquitinase (DUB) removes ubiquitin from substrate and represses the degradation of protein (13). DUBs can be divided into six families: ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs),

Machado - Josephin domain-containing proteases (MJDs), ovarian tumour proteases (OTUs), motif-interacting with ubiquitin-containing novel DUB family (MINDYs) and JAB1, MPN, MOV34 family (JAMMs). Among DUBs, USP41 covered in this study is a cysteine protease (13). USPs are abnormally expressed in various diseases such as cancer. For example, overexpression of USP29 stabilizes Snail and is associated with stemness and drug tolerance in lung cancer (14). USP25 induces proliferation of leukemia cells via stabilization of tyrosine kinase BCR-ABL that is responsible for STAT5 activation (15). Thus, DUB may play a role as a crucial molecular target for cancer. USP41 is highly expressed in breast cancer and promotes cancer development via Receptor for Activated C Kinase 1 (RACK1) (16). Ji et al. reported that USP41 was highly expressed in lung cancer patients and associated with low survival of lung cancer (17).

Although USP41 promotes the proliferation and invasion of cancer cells, underlying mechanism is still unclear. In this study, I aimed to understand how USP41 enhances EMT and to elucidate the molecular mechanism of USP41-mediated Snail stabilization in human breast cancer cells.

2. Materials and Methods

2.1. Cell Lines and Cell Culture:

Human breast carcinoma cells (MDA-MB-231, MDA-MB-361) were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were maintained in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% fetal bovine serum (Welgene, Gyeongsan, Korea), 1% antibiotic-antimycotic (Gibco, Waltham, Massachusetts, USA) and 0.2% gentamicin (Thermo Fisher Scientific, Waltham, MA, USA). The cells were cultured at 37 ℃, 5% CO_2 in humidified air.

2.2. Antibodies and Reagents:

Thermo Fisher Scientific supplied anti-USP41 antibody (Waltham, MA, USA). Anti-Snail, anti-E-cadherin, anti-Vimentin and anti-Slug antibodies purchased from Cell signaling (Beverly, MA, USA) were used for western blotting. Anti-Actin antibody, cycloheximide and MG132 supplied by Sigma Chemical Co. (St. Louis, MO, USA) were used for experiment. Enzo Life Sciences supplied anti-Ub antibody (San Diego, CA, USA). Santa Cruz Biotechnology supplied protein G agarose bead (St. Louis, MO, USA). TriZol was purchased from Life Technologies (Gaithersburg, MD, USA). Immobilon western chemiluminescent HRP substrate was supplied by Merck Millipore (Burlington, MA, USA).

2.3. Transfection:

Control siRNA and USP41 siRNA obtained from Bioneer (Daejeon, Korea) were used with Lipofectamine® RNAiMAX Reagent (Invirtrogen, Calshad, CA, USA) for siRNA transfection. pcDNA 3.1 vector was obtained from Invitrogen (Carlsbad, CA, USA). pCMV-USP41-FLAG plasmid was obtained from life science market (Dundas St, Mong Kok, HK). For plasmid transfection, Lipidofect-P was used (Lipidomia, Seongnam, Korea).

2.4. Western Blotting:

Cells were dissolved in RIPA lysis buffer supplemented with 20 mM HEPES and 0.5% Triton X-100, pH 7.6. Proteins were separated and transferred by electrophoresis to the nitrocellulose membranes (GE Healthcare life Science, Pittsburgh, PO, USA). The membranes were blocked with 5% non-fat milk containing 0.02% sodium azide, and then reacted with a primary and secondary antibody step by step. After washing with 1 X tris buffered saline with tween, images of protein band were captured by immobilon western chemiluminescent HRP substrate and iBright™ Imager (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Colony Formation:

After seeding at 6 well plate, cells were incubated for 3 days. Then, after washing with phosphate buffered saline (PBS), cells were stained with 0.25% crystal violet for 5 min at room temperature. After washing with PBS, the buffer was removed.

2.6. Transwell:

Cells of 1 x 10^5 were seeded in the upper part of transwell with DMEM and the bottom of the transwell with DMEM containing 1% FBS. After 24 h incubation, cells were fixed with methanol at -20 °C for 10 min and washed with distilled water for twice. And top surface cells of the transwell were removed using a cotton swab. Cells that transferred to under the surface of the transwell were stained 0.25% crystal violet for 20 min and washed with distilled water.

2.7. Wound Healing Assay:

After seeding in 12 well plate for 24h, the cells were scratched by a yellow tip and the medium was changed to DMEM containing 1% FBS.

2.8. In Vitro Ubiquitination Assay:

Cells were co-transfected with HA-UB plasmid and control siRNA or USP41 siRNA using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). The transfected cells were treated with MG132. After harvest, cells were washed with PBS containing 10 mM N-ethylmaleimide (NEM), resuspended in PBS containing 10 mM NEM. 1% SDS was added to the lysates and boiled at 95 °C for 10 min. RIPA lysis buffer involving 1 mM PMSF, 1 mM NA_3VO_4, 0.01 μg/mL leupeptin, 2 mg/mL aprotinin and 5 mM NEM were added to lysates and the lysates were dissolved using syringe and centrifuged 13,000 RPM for 15 min at 4 °C. The supernatants reacted with the primary antibody overnight and added protein G agarose bead for 2 h. After centrifuging, the lysates

were washed with RIPA lysis buffer and boiled after adding 2 × cooking buffer for 10 min. After centrifuging for 10 min, western blotting was performed. Ubiquitination was identified in denaturation condition with anti-Ub antibody.

2.9. Immunoprecipitation (IP) Assay:

After the harvest, cells were washed with PBS and dissolved using RIPA buffer. After sonication, the cells were centrifuged and the supernatants reacted with primary antibody overnight. After adding protein G agarose beads to the lysates, it reacted. After centrifuging, the supernatants were removed. Lysates were washed with RIPA lysis buffer, and then boiled adding the 2 × cooking buffer. The interaction between the protein was identified by western blotting.

2.10. Quantitative PCR (qPCR):

cDNA was produced using RNA extracted by TriZol and M-MLV reverse transcriptase (Gibco-BRL, Gaithersburg, MD, USA). After dilution of cDNA, it reacted with SYBR Fast qPCR Mix (Takara Bio Inc., Shiga, Japan) and primers. For qPCR analysis, Thermal Cycler Dice® Real-Time System III (Takara Bio Inc., Shiga, Japan) was used. The primers were used as follows; Actin (forward) 5′-CTA CAA TGA GCT GCG TGT G-3′ and (reverse) 5′-TGG GGT GTT GAA GGT CTC-3′; USP41 (forward) 5′-TGG AGG GCA GTA TGA GCT TTT T-3′ and (reverse) 5′-ATG ACC GGA GTC TGC CAT TC-3′; and Snail (forward) 5′-TTT CTG GTT CTG TGT CCT CTG CCT-3′ and (reverse) 5′-TGA GTC TGT CAG CCT TTG TCC

TGT-3′.

2.11. Statistical Analysis:

The data were analyzed by a one-way ANOVA and post hoc comparisons (Student-Newman-Keuls) using the Statistical Package for Social Sciences 22.0 software supplied from SPSS Inc. (Chicago, IL, USA).

3. Results

3.1. USP41 Knockdown Inhibits Cell Migration and Invasion in Breast Cancer Cells:

To determine whether USP41 regulates the migration ability of breast cancer cells, USP41 was knockdown in breast cancer cell lines. The mRNA and protein expression of USP41 were downregulated by USP41 knockdown (Figure 1A). Compared with control, knockdown of USP41 inhibited the wound healing ability (Figure 1B). To identify the role of USP41 in invasion, transwell assay was performed. USP41 knockdown significantly suppressed the ability of invasion in MDA-MB-231 and MDA-MB-361 cells (Figure 1C). These results indicate that USP41 knockdown inhibits the migration and invasion of breast cancer cells.

3.2. USP41 Knockdown Inhibits the Proliferation of Breast Cancer Cells:

To examine the role of USP41 in proliferation, colony formation assay and growth rate analysis were performed. Knockdown of USP41 inhibited colony formation and cell growth of MDA-MB-231 and MDA-MB-361 cells (Figure 2A&B). These data suggest that USP41 is associated with cell proliferation of breast cancer cells.

3.3. Ectopic Expression of USP41 Induces Cell Growth and Invasion in Breast Cancer Cells:

To further examine the functional significance of USP41 in tumor progression, I used USP41 overexpression in MDA-MB 231 and MDA-MB 361 cells. Overexpression efficiency at the protein level was observed by western blotting (Figure 3A). Ectopic expression of USP41 accelerated cell growth (Figure 3B). In addition to, overexpression of USP41 improved the invasion ability (Figure 3C). Overall, these data suggest that USP41 overexpression could enhance the growth and invasion of breast cancer cells.

3.4. The effect of USP41 siRNA on the expression levels of EMT related proteins:

To investigate the mechanism of USP41-mediated EMT, breast cancer cells were transfected using control siRNA or USP41 siRNA with increasing concentration. USP41 knockdown markedly dose-dependently reduced Snail expression. However, Vimentin, E-cadherin and Slug were not changed in both cell lines (Figure 4). These data suggest that the expression level of USP41 is associated with the expression level of Snail.

3.5. Ectopic Expression of USP41 Upregulates Snail Protein Expression:

To further investigate the protein expressional correlation between USP41 and Snail, USP41 was overexpressed in MDA-MB-231 and MDA-MB-361 cells. Ectopic expression of USP41 induced the upregulation of Snail protein (Figure 5). These data suggest that the

expression of USP41 has a positive correlation with the Snail expression.

3.6. USP41 Regulates Snail Expression at Post-translational Level:

To elucidate the mechanism of USP41 inhibition-induced Snail downregulation, I used the Snail mRNA levels. After transfection with USP41 siRNA, mRNA level of Snail was identified using qPCR. USP41 knockdown did not alter the mRNA level of Snail in MDA-MB-231 and MDA-MB-361 cells (Figure 6A). Thus, I investigated whether USP41 knockdown modulates Snail at the post-translational level. MDA-MB-231 and MDA-MB-361 cells transfected with control siRNA or USP41 siRNA were time-dependently treated with cycloheximide (CHX: protein synthesis inhibitor). USP41 siRNA markedly accelerated Snail degradation (Figure 6B). Furthermore, proteasome inhibitor MG132 reversed the USP41 siRNA-mediated downregulation of Snail in MDA-MB-231 and MDA-MB-361 cells (Figure 6C). These results suggest that USP41 stabilizes Snail through inhibition of proteasome-mediated degradation.

3.7. USP41 Interacts and Stabilizes with Snail:

Considering that USP41 induced Snail expression, I further investigated whether USP41 interacts with Snail. After immunoprecipitation with Snail antibody, and USP41 protein level was determined in MDA-MB-231 and MDA-MB-361 cells. The interaction between endogenous USP41 and Snail was identified in MDA-MB-231

and MDA-MB-361 cells (Figure 7A). To further examine whether USP41 has an effect on endogenous Snail ubiquitination, I transfected control or USP41 siRNA into MDA-MB-231 and MDA-MB-361 cells. USP41 siRNA elevated the ubiquitination levels of Snail in both cells (Figure 7B). These results suggest that USP41 is crucial for the stability of Snail.

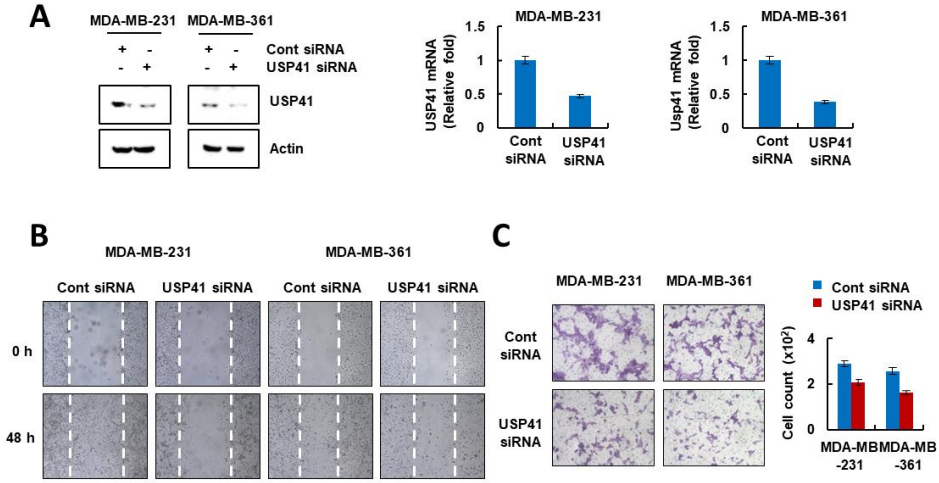

Figure 1. USP41 knockdown inhibits cell migration and invasion in breast cancer cells. (A-C) MDA-MB-231 and MDA-MB-361 cells were transfected using control siRNA (Cont siRNA) or USP41 siRNA. (A) The protein and mRNA expression levels of USP41 were examined by western blotting and q-PCR, respectively. (B) Migration ability was examined by wound healing assay. (C) Invasion ability was examined by transwell assay. The values in the graph represent the mean ± SD of two and three independent samples, respectively. $p^* < 0.01$ compared to the control.

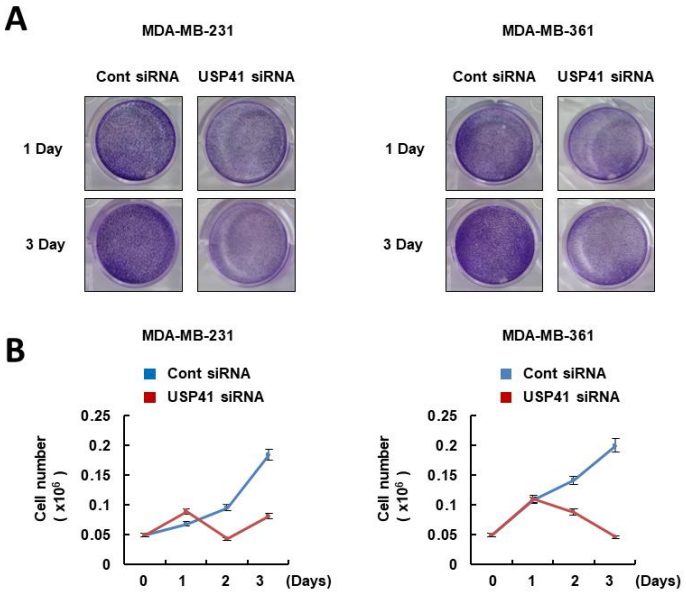

Figure 2. USP41 knockdown inhibits the proliferation of breast cancer cells. (A-B) MDA-MB-231 and MDA-MB-361 cells were transfected with Cont siRNA or USP41 siRNA. Colony formation and cell growth were determined for the indicated time points. The values in the graph represent the mean ± SD of three independent samples, respectively. $p^* < 0.01$ compared to the control.

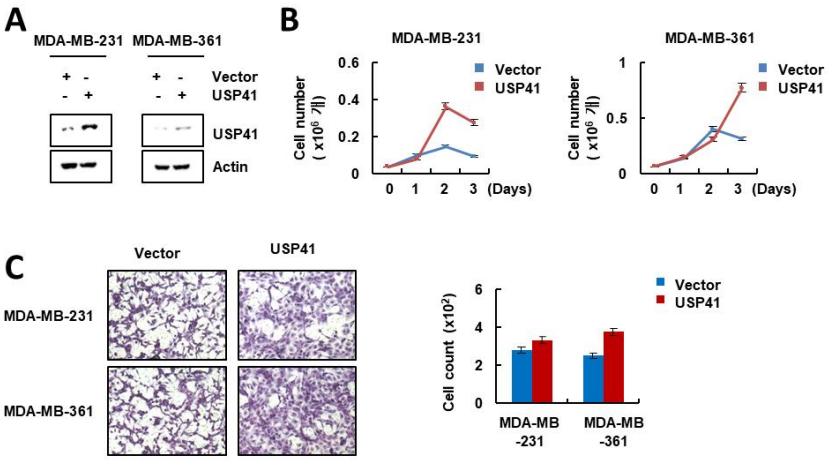

Figure 3. Ectopic expression of USP41 induces cell growth and invasion in breast cancer cells. (A-C) MDA-MB-231 and MDA-MB-361 cells were transfected using pcDNA3.1(+) (Vector) or USP41 expression plasmid. (A) Protein expression was examined by western blotting. (B) Cell growth was determined for the indicated time points. (C) Invasion ability was examined by transwell assay. The values in the graph represent the mean ± SD of three independent samples, respectively. $p^{*} < 0.01$ compared to the control.

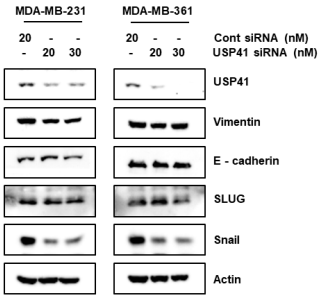

Figure 4. The effect of USP41 siRNA on the expression levels of the EMT related proteins. Cont siRNA 20 nM or USP41 siRNA 20 nM and 30 nM were used for transfection in MDA-MB-231 and MDA-MB-361 cells. The protein expression were determined by western blotting. EMT: epithelial-mesenchymal transition.

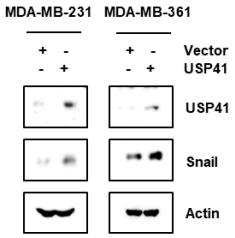

Figure 5. Ectopic expression of USP41 upregulates Snail protein expression. MDA-MB-231 and MDA-MB-361 cells were transfected with Vector or USP41 expression plasmid. The expression levels of USP41 and Snail were determined by western blotting.

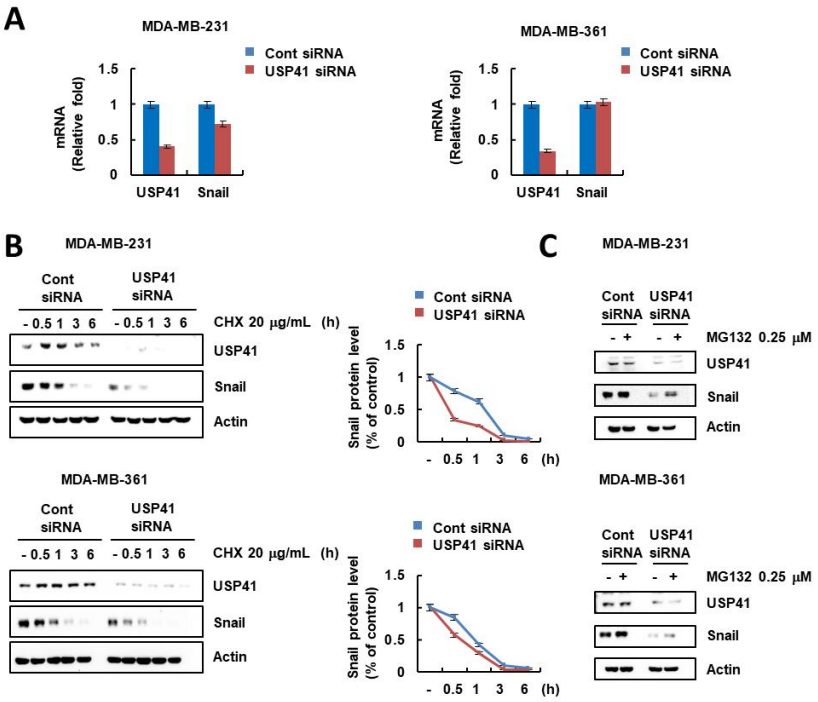

Figure 6. USP41 regulates Snail expression at post-translational level. (A-C) MDA-MB-231 and MDA-MB-361 cells were transfected with Cont siRNA or USP41 siRNA. (A) The mRNA levels of USP41 and Snail were measured by q-PCR. (B) MDA-MB-231 and MDA-MB-361 cells were transfected with Cont or USP41 siRNA, and then treated 20 μg/mL cycloheximide (CHX) for the indicated time points. The density of Snail protein band was quantified by Image J. (C) After 48 h transfection, MDA-MB-231 and MDA-MB-361 cells were treated with or without 0.25 μM MG132 for 12 h. (B-C) Protein expression was determined by western blotting. The values in the graph represent the mean ± SD of two or three independent samples. $p^{*} < 0.01$ compared to the control.

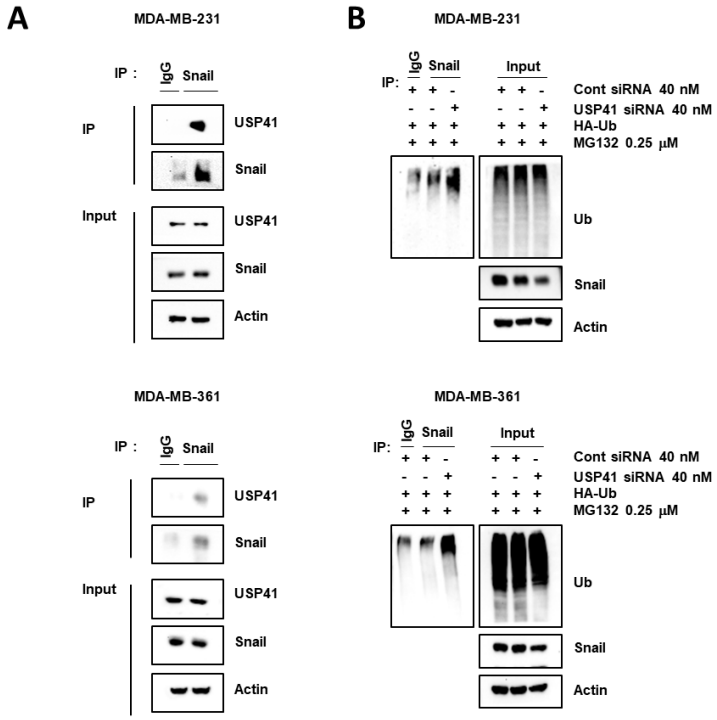

Figure 7. USP41 interacts and stabilizes with Snail. (A) Interaction between endogenous Snail and USP41 was examined by immunoprecipitation assay using the Snail antibody. (B) Ubiquitination of Snail was identified using ubiquitination assay. MDA-MB-231 and MDA-MB-361 cells were co-transfected with Cont siRNA or USP41 siRNA and HA-Ub plasmid. Then, the cells were treated 0.25 μM MG132 for 24 h. Snail ubiquitination and protein expression were detected by western blotting.

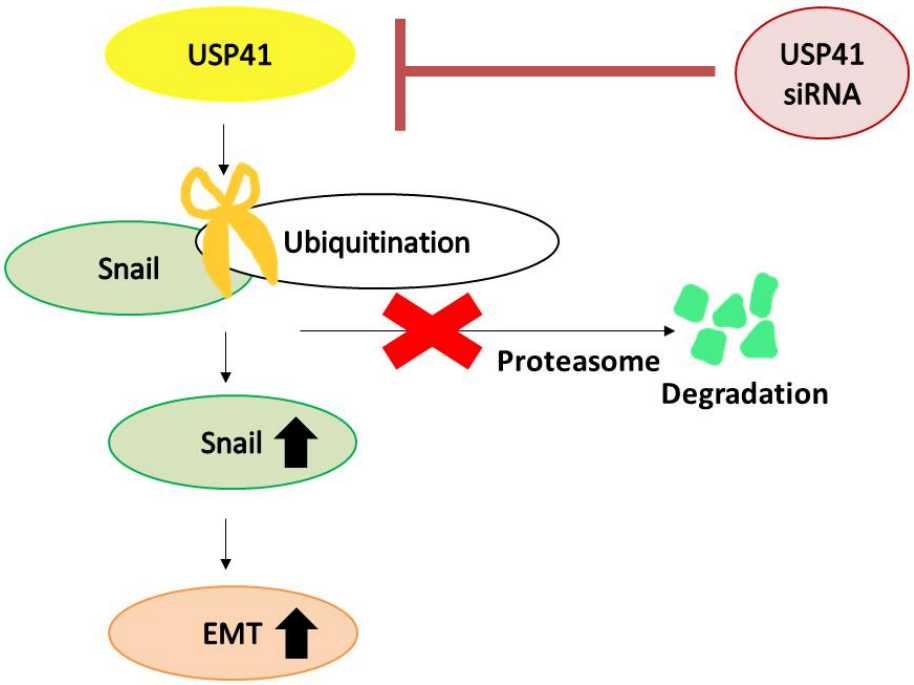

Figure 8. The scheme of the mechanism about USP41-mediated Snail stabilization. USP41 interacts with Snail and decreases the ubiquitination of Snail. Therefore, upregulated Snail promotes EMT progression.

4. Discussion

In this study, I suggest that USP41 stabilized Snail protein and enhanced cell growth and metastasis in breast cancer cells. USP41 interacted with Snail, resulting in Snail deubiquitination and stabilization of Snail. siRNA-mediated USP41 knockdown inhibited wound healing, cell migration, and cell growth through Snail downregulation. Therefore, USP41 plays a critical role in Snail-mediated EMT progression.

Li et al. reported that USP41 is one of the eight prediction mRNAs for breast cancer prognosis (18). USP41 was overexpressed in lung cancer patients. Interestingly, short hairpin RNA-mediated USP41 downregulation induced upregulation of E-cadherin in lung carcinoma A549 cells (17). However, USP41 siRNA had no effect on E-cadherin in MDA-MB-231 and MDA-MB-361 cells (Figure 4). The different result is probably due to different cancer cell lines. It was required to find out through more experiments. Huang et al. reported that ectopic expression of USP41 markedly induced proliferation and migration in breast carcinoma cells such as MDA-MB-231 and MCF-7 (16). Although USP41 did not show a direct deubiquitination result, it interacted with RACK1 and USP41 knockdown showed decreased expression of RACK1. Moreover, further studies are needed to determine whether RACK1 affects USP41-mediated Snail deubiquitination.

EMT progression is triggered during metastasis and caused a death rate in cancer patients (19). Snail, a key transcription factor in the EMT process, is a crucial factor in tumor metastasis (11). The E3 enzymes are involved in the degradation of Snail (20-22) but a lot of research on DUBs has not been conducted. Thus, DUBs are now emerging as new modulators for Snail. Zhou et al. reported that OTUB1 enhances the

metastasis of esophageal cancer by Snail protein stabilization (23). DUB3 also interacts with and stabilizes Snail. Additionally, the specific DUB3 inhibitor, WP1130, inhibited DUB3-mediated Snail stabilization in vivo (24). In esophageal cancer, EIF3H and PSMD14 promoted metastasis through stabilization of Snail (25,26). My results indicate that USP41 is a key DUB in Snail stabilization.

Collectively, this study shows that USP41 inhibition suppresses metastasis through Snail downregulation. Therefore, USP41 could be used as a crucial target for cancer therapy, given its role in the inhibition of metastasis of breast cancer.

5. Summary

USP41 is overexpressed in multiple tumors including lung and breast tumor. However, the detailed molecular mechanism and role of USP41 in EMT of breast cancer are poorly understood. USP41 knockdown inhibits cell proliferation, wound healing, and invasive ability of breast cancer cells. These effects are enhanced by overexpression of USP41. USP41 knockdown induces the downregulation of Snail protein expression. Furthermore, USP41 interacts with and stabilizes Snail. My study reveals a critical role of USP41 in EMT and metastasis, and provides a crucial target against breast cancer therapy.

References

1. Kalluri R, Weinberg RA: The basics of epithelial-mesenchymal transition. J Clin Invest 2009; 119: 1420 - 28.

2. Du B, Shim JS: Targeting Epithelial - Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. Molecules 2016; 21: 965.

3. Debnath P, Huirem RS, Dutta P, Palchaudhuri S: Epithelial - mesenchymal transition and its transcription factors. Biosci Rep 2022; 42: BSR20211754.

4. Vu T, Datta PK: Regulation of EMT in Colorectal Cancer: A Culprit in Metastasis. Cancers 2017; 9: 171.

5. Odero-Marah V, Hawsawi O, Henderson V, Sweeney J: Epithelial-Mesenchymal Transition (EMT) and Prostate Cancer. Adv Exp Med Biol 2018; 1095: 101 - 10.

6. Tang X, Sui X, Weng L, Liu Y: SNAIL1: Linking Tumor Metastasis to Immune Evasion. Front Immunol 2021; 12: 724200.

7. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M et al. : Dual Regulation of Snail by GSK-3beta-Mediated Phosphorylation in Control of Epithelial-Mesenchymal Transition. Nat Cell Biol 2004; 6: 931 - 40.

8. Zhang W, Feng M, Zheng G, Chen Y, Wang X, Pen B et al. : Chemoresistance to 5-fluorouracil induces epithelial - mesenchymal transition via up-regulation of Snail in MCF7 human breast cancer cells. Biochem Biophys Res Commun 2012; 417: 679-85.

9. Haslehurst AM, Koti M, Dharsee M, Nuin P, Evans K, Geraci J et al. : EMT transcription factors snail and slug directly contribute to cisplatin resistance in ovarian cancer. BMC cancer 2012; 12: 1-10.

10. De Las Rivas J, Brozovic A, Izraely S, Casas-Pais A, Witz IP, Figueroa A: Cancer drug resistance induced by EMT: Novel therapeutic strategies. Arch Toxicol 2021; 95: 2279-97.

11. Wang Y, Shi J, Chai K, Ying X, P Zhou B: The role of snail in EMT and tumorigenesis. Curr Cancer Drug Targets 2013; 13: 963-72.

12. Suresh B, Lee J, Kim K-S, Ramakrishna S: The Importance of Ubiquitination and Deubiquitination in Cellular Reprogramming. Stem Cells Int 2016; 2016: 6705927.

13. Harrigan JA, Jacq X, Martin NM, Jackson SP: Deubiquitylating enzymes and drug discovery: emerging opportunities. Nat Rev Drug Discov 2018; 17: 57 - 78.

14. Wu Y, Zhang Y, Wang D, Zhang Y, Zhang J, Zhang Y et al. : USP29 enhances chemotherapy-induced stemness in non-small cell lung cancer via stabilizing Snail1 in response to oxidative stress. Cell Death Dis 2020; 11: 1-14.

15. Shibata N, Ohoka N, Tsuji G, Demizu Y, Miyawaza K, Ui-Tei K et al. : Deubiquitylase USP25 prevents degradation of BCR-ABL protein and ensures proliferation of Ph-positive leukemia cells. Oncogene 2020; 39: 3867-78.

16. Huang M, Xiao J, Yan C, Wang T, Ling R: USP41 promotes breast cancer via regulating RACK1. Ann Transl Med 2021; 9: 1566.

17. Ji J, Yang S, Zu L, Li Y, Li Y: Deubiquitinating enzyme USP41 promotes lung cancer cell proliferation and migration. Thorac Cancer 2021; 12: 1041-7.

18. Li H, Gao C, Zhuang J, Liu L, Yang J, Liu C et al. : An mRNA characterization model predicting survival in patients with invasive breast cancer based on The Cancer Genome Atlas database. Cancer Biomark 2021; 30: 417-28.

19. Francou A, Anderson KV: The Epithelial-to-Mesenchymal Transition (EMT) in Development and Cancer. Annu Rev Cancer Biol 2020; 4: 197-220

20. Viñas-Castells R, Beltran M, Valls G, Gómez I, García JM, Montserrat-Sentís B et al. : The Hypoxia-controlled FBXL14 Ubiquitin Ligase Targets SNAIL1 for Proteasome Degradation. J Biol Chem 2010; 285: 3794-805.

21. Jin Y, Shenoy AK, Doernberg S, Chen H, Luo H, Shen H et al. : FBXO11 promotes ubiquitination of the Snail family of transcription

factors in cancer progression and epidermal development. Cancer Lett 2015; 362: 70-82.

22. Ruan L, Liu W, Yang Y, Chu Z, Yang C, Yang T et al. : TRIM16 overexpression inhibits the metastasis of colorectal cancer through mediating Snail degradation. Exp Cell Res 2021; 406: 112735.

23. Zhou H, Liu Y, Zhu R, Ding F, Cao X, Lin D et al. : OTUB1 promotes esophageal squamous cell carcinoma metastasis through modulating Snail stability. Oncogene 2018; 37: 3356-68.

24. Wu Y, Wang Y, Lin Y, Liu Y, Wang Y, Jia J et al. : Dub3 inhibition suppresses breast cancer invasion and metastasis by promoting Snail1 degradation. Nat Commun 2017; 8: 1-6.

25. Zhu R, Liu Y, Zhou H, Li L, Li Y, Ding F et al. : Deubiquitinating enzyme PSMD14 promotes tumor metastasis through stabilizing SNAIL in human esophageal squamous cell carcinoma. Cancer Lett 2018; 418: 125-34.

26. Guo X, Zhu R, Luo A, Zhou H, Ding F, Yang H et al. : EIF3H promotes aggressiveness of esophageal squamous cell carcinoma by modulating Snail stability. J Exp Clin Cancer Res 2020; 39: 1-15.

USP41 Enhances Epithelial-mesenchymal Transition of Breast Cancer Cells through Snail Stabilization

Yoon, Ji Yun

Department of Immunology
Graduate School

Keimyung University

(Supervised by Professor Kwon, Taeg Kyu)

(Abstract)

Ubiquitination, one of post-translational modification, causes proteasome-mediated protein degradation by attaching ubiquitin to target proteins. Deubiquitinase reverses the ubiquitination pathway by removing ubiquitin chain to protein, inducing of protein stability. Multiple cancers have high expression of USP41. And USP41 is involved in invasion, apoptosis and drug resistance. However, the detailed mechanism and role of USP41 in breast cancer have not been elucidated. Knockdown of USP41 inhibited migration and growth in breast cancer cells, whereas overexpression of USP41 increased cell growth and invasion. Furthermore, depletion of USP41 downregulated Snail protein expression, an epithelial-mesenchymal transition marker. USP41 directly interacted with Snail and inhibited ubiquitination of Snail, increasing of

stabilization. USP41 knockdown-mediated Snail downregulation was reversed by proteasome inhibitor (MG132). Therefore, these data demonstrate that USP41 induces invasion through Snail stabilization in breast cancer cells.

유방암에서 USP41의 상피-중간엽 전이 조절

윤지윤
계 명 대 학 교 대 학 원
의학과 면역학 전공
(지도교수 권 택 규)

(초록)

번역 후 수정 과정 중 하나인, 유비퀴틴화는 단백질에 유비퀴틴을 부착시킴으로써 프로테아좀을 매개로 한 단백질 분해를 야기시킨다. 탈유비퀴틴화 효소는 유비퀴틴 사슬을 제거함으로써 유비퀴틴화 신호를 반전시켜 결과적으로 단백질의 안정성을 유도한다. 탈유비퀴틴화 효소인 USP41은 많은 암에서 발현이 높으며 침습, 증식, 세포사멸, 약물 내성과도 관련이 있다. 그러나 유방암에서 상피-중간엽 전이에 대한 USP41의 자세한 메커니즘은 여전히 불분명하다. 본 연구에서는, 유방암에서의 USP41의 역할을 밝히고 상피-중간엽 전이 유도에 관여하는 메커니즘을 규명하였다. USP41의 억제는 유방암 세포의 이동과 성장을 억제시킨 반면, USP41의 과발현은 이동을 증가시켰다. 이는 상피-중간엽 전이 주요 조절자인 Snail로 매개되는 것이며, USP41은 Snail과 상호작용하여 Snail의 유비퀴틴화를 억제하여 안정성을 증가시킨다는 것을 확인하였다. 따라서 USP41이 Snail을 통해서 상피-중간엽 전이를 증가시키며, 유방암 치료의 새로운 분자 타겟이 될 수 있음을 제

시한다.