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석사학위 논문

# The functional role of alternative splicing variants in cancer progression

조선대학교 대학원

의과학과

설 예 원

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# The functional role of alternative splicing variants in cancer progression

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이 논문을 석사학위신청 논문으로 제출함

2017 년 10 월

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의과학과

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# 설예원의 석사학위논문을 인준함

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# CONTENTS

## RESULTS

## DISCUSSION



## ABSTRACT



## REFERENCES

# CONTENTS OF FIGURES

# 국문초록

## 선택적 접합 변이체가 암진행에 미치는 영향

설 예 원

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선택적 스플라이싱은 pre-mRNA 프로세싱은 유전자 발현의 중요한 조절 인자이다. 왜냐하면 exon이 남아있고 intron이 제거 된 유전 정보의 사용에 대한 충분한 복사본을 생산하기 때문이다. 따라서 선택적 스플라이싱은 유전자 조절과 유전적 다양성을 위한 중요한 메커니즘이다.

Jumonji Domain Containing6 (JMJD6) 는 histone 4의 arginine 3 과 histone

3의 dimethylase된 arginine2의 메틸기를 우선적으로 제거함으로써 전사의 동적 조절을 가능하게 하는 histone arginine dimethylase 이다. JMJD6는 또한 RNA splicing 을 조절함으로써 유전자 발현을 조절하여 JMJD6가 다각적인 후성 형성 조절자임을 시사한다. 임상적으로, JMJD6의 과발현은 폐 및 유방을 포함한 다양한 인간 암에서 나쁜 예후와 밀접하게 관련되어 있다. JMJD6는 암 줄기세포(CSC, 암 유발 세포로 알려짐) 특성으로 잘 알려진 두 가지 표현형이 migration/invasion 및 혈관 신생 발아를 촉진시키는 것으로 보고되어 있다.

최근 연구들은 선택적 스플라이싱이 암을 비롯한 다양한 질병과 밀접하게 관련되어있다고 보고되어있다. 그러나 암의 진행에 따른 기능적 결과는 아직 밝혀지지 않았다. 우리는 암 데이터베이스 분석을 통해 암 관련 JMJD6 전사 변이형을 발견했다. 이 연구에서, 우리는 JMJD6의 두 가지 별개 단백질의 암 조직에서의 차별적인 발현 변화, 즉 정상 조직과 비교하여 암에서 절단된 JMJD6 mRNA의 발현이 증가하는 것을 확인하였다. 우리는 선택적 스플라이싱에 의해 절단된 JMJD6 mRNA가 주로 암과 관련이 있음을 시사한다.

# INTRODUCTION

Alternative splicing is a vital molecular affair in the gene expression process. Alternative splicing is a key process that regulates pre-mRNA maturation. Splicing occurs when a group of proteins and RNP recognize specific RNA sequences maintained at the boundaries of introns.[1] The splicing reaction is carried out by a multi-protein / RNA complex called a spliceosome consisting of five small-nuclear ribonucleoproteins (snRNPs), U1, U2, U4, U5 and U6.[2] Spliceosome recognize intron-exon boundaries, and removes interventions introns through two transesterification reactions, this reaction results in ligation of two adjacent exons.[3] During this process, consists in appreciate of exons and introns, exons being remain together and introns being eliminated. [4] Different modes of alternative splicing are illustrated and we distinguish it from the initiation transcriptional alternative. (Figure 1) Constitutive or cassette exon is an exon may be spliced out of the primary transcript of retained. This is the most

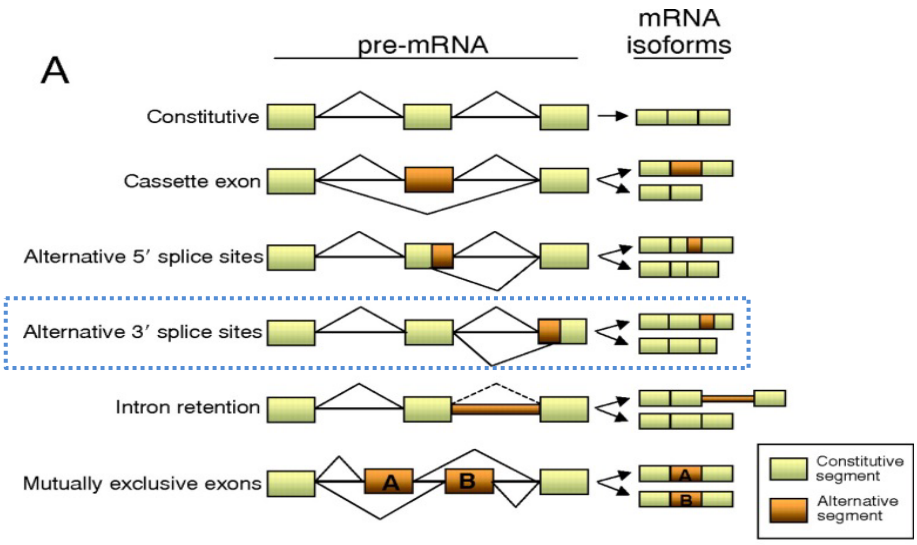



**Figure 1 Different modes of alternative splicing.**

common mode in mammalian pre-mRNAs.[5] Alternative 5' splice sites and Alternative 3' splice sites are a mode in which another splice junction (receptor site) is used to change the boundary of the downstream exon. Intron retention is the least common mode in mammals. This mode can be distinguished from exon skipping because the sequence it holds is not the position of the intron. Mutually exclusive exon is a mode in which only one mRNA is retained after splicing among two exons. We will discuss the alternative splicing in the alternative acceptor site (blue box) of the various modes mentioned above. human multi-exon

genes are approximately that up to 95% alternatively spliced.[6] Alternative pre-mRNA processing is a key regulator of gene expression as it generates plentiful transcripts the use of genetic information.[7] Thus, Alternative splicing is one of the important mechanisms for gene regulation and gene diversity. Alternative splicing is regulatory proteins play decisive roles in regulating alternative splicing, associated with physiological processes and cell development programs. The disruption of the alternative splicing likely affects cell differentiation, resulting in the development of cancer and other diseases.[8]

One of the major causes of disease is defected of mRNA splicing. At least 15% of all disease-causing single base pair mutations affect the splicing. Therefore, the splicing process and its control are very important.[9-11] The relationship between alternative splice forms and cancer is well established.[12] Alternative splicing is newly become a hallmark for cancer and the target for the evolution of new therapeutic molecules.[13] The concentration and activation of splicing fac-

tors may be affected in cancerous processes, which involve the production of different isoforms and, subsequently, the alteration of the cellular function.[14] Several protein isoforms generated by alternative splicing are crucial for cancer progression and are the issue of experimental therapeutic intervention.[15]

Jumonji domain containing 6 (JMJD6) is a member of the JmjC domain-containing family of proteins that participate in a wide range of oxidation reactions.[16] The JmjC domain containing enzymes catalyzes demethylation through an oxidative reaction that depends on two cofactors, iron Fe(Ⅱ) and $\alpha$-ketoglutarate.[17] Specifically, these enzymes catalyze the hydroxylation of mono-, di- or trimethylated lysine in histone tails, giving rise to an unstable hydroxyl-methyl group, which is released spontaneously as formaldehyde.[16] Some JmjC domain-containing histone demethylases have been linked to cancer.[18] However, the mechanisms of their oncogenic activities are not well understood.

JMJD6 was first identified as a phosphatidylserine receptor on cell membrane functioning in phagocytosis of apoptotic cells. Subsequently, it was recognized that JMJD6 possesses catalytic activity as dioxygenase in the nucleus.[19] JMJD6 has been shown to interact with U2AF65, to catalyse hydroxylation of lysine residues in U2AF65 and to regulate alternative splicing.[20] The biological importance of JMJD6 in pre-mRNA splicing was demonstrated in mouse endothelial cells where JMJD6 knockdown changed splicing of the vascular endothelial growth factor (VEGF)-receptor Flt I pre-mRNA and thereby promoted expression of a soluble form of the receptor, which inhibits angiogenesis by binding to VEGF.[21] It has also been reported that JMJD6 affects cell scattering and motility. The degree of scattering is a sign of cell motility. The degree of scattering is a sign of cell motility and epithelial-mesenchymal transformation (EMT), and overexpression of JMJD6 increases the extent of breast cancer cell scattering.[22] Moreover, JMJD6 is overexpressed in and related to a poor

prognosis of cancers. JMJD6 is divided into long forms and short form through Alternative splicing. However, the JMJD6 mRNA variant by alternative splicing was unknown about the molecular pathways of cancer progression. Alternative splicing of JMJD6 produced the two types of transcript variants that full-length JMJD6 mRNA (JMJD6 LF) and truncated mRNA (JMJD6 SF). In this study, we investigated the different roles of the truncated JMJD6 mRNAs JMJD6LF and JMJD6SF.

# MATERIALS AND METHODS

## 1. Cell culture and treatment

Human breast carcinoma cell lines, MCF7, MDA-MB-231 were cultured in RPMI1640 medium (WelGene). supplemented with 10 % fetal bovine serum(FBS) and penicillin streptomycin (0.1 mg/ml). Cell growth was monitored under an inverted microscope. Upon reaching 70-90 % confluency, cells were digested with 0.5 % trypsin-EDTA before being passaged. All cell lines were maintained at 37 ° C and in 5 % CO2 at saturated humidity. Cells in exponential growth were harvested for subsequent experiments.

## 2. Plasmid DNA preparation

Plasmid DNA was prepared by purchasing JMJD6 LF (NM_001081461) and JMJD6 SF (NM_015167) Human ORF cDNA Clone. (ORIGENE, USA)

## 3. Small interfering RNA(siRNA) transfection

The target sequences were 5′- CAC CUG UAA UCC CAG CAC UUU -3′ for

JMJD6 long form, 5′- CAC GGG AAC CCA UUC ACU UAG -3′ for JMJD6 short form. Which was introduced using Lipofectamine RNAimax(Invitrogen)in Opti-MEM medium. MCF-7 cells were plated in 60-mm dishes and transfected with 50nM siRNA. The harvested were harvested after 48hr.

## 4. RNA isolation

Following cell lysis with the TRIzol lysis reagent. After the addition of chloroform (Merck Millipore), the homogenate was separated into aqueous and organic phases by centrifuge at 13,000g for 30 min. RNA was precipitated from the aqueous phase by the addition of isopropanol (Merck Millipore) and incubation at -20℃ for overnight. The pellet was then washed 70% ethanol (Merck Millipore), air dried, and dissolved in 30㎕ of RNase-free water.

## 5. Real-Time PCR

Real-Time quantitative PCR was performed using the SYBR Premix Ex Taq (Takara). cDNA of MCF7 cells by PCR using the JMJD6 primers 5′- GGT TGC

TGG TGC AAA TGC TT -3′ (Long form Forward primer), 5′- CTG GGG CAT CCT TAT ACG CC -3′ (Long form Reverse primer) and 5′- GTC TGT ATG GAA GGA CAC GCT -3′ (Short form Reverse primer), 5′- AAA GCT ACT GGA GCA AAC GC -3′ (Short form Reverse primer). The PCR reaction consisted of 12.5 ㎕ of SYBR Green Master Mix, 1㎕ of forward and reverse primers, and 0.5㎕ of template cDNA in a total volume of 20 ㎕. Cycling was performed using the default conditions of the Mxpro software : 95℃ 10min ,( 95℃ 30sec, 62℃ 1min ) X30 Cycles, 95℃ 1min, 55℃ 30sec, 95℃ 30sec. Relative RNA expres-sion levels were quantified using the ⊿⊿CT method.

## 6. RT-PCR

The cDNA products were then used as PCR templates to amplify its target genes. PCR amplification using 1㎍ of CDNA aliquots were performed by adding 2.5 mM dNTPs, Taq DNA polymerase, and 10 pmol each of forward and reverse primers in a PCR buffer. Reactions were processed in a DNA Termal Cycler

(Eppendorf, USA) through 30 cycles of 1 min of denaturation at 95℃, 1 min of annealing at 62℃, 1min of extension at 72℃. PCR products were electrophoresed on a 1.8% agarose gel, and visualized using a Gel Imaging Systems with UV transilluminator (Thomas Scientific, USA). The band densities were quantified using Image-J program.

## 7. Western blot analysis

Cells were washed with 1x PBS and lysed in RIPA buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 % NP40, 0.5 % sodium deoxycholate(NaDOC), 0.1 % sodium dodecyl sulfate(SDS), 1 % TritonX-100) with protease inhibitors (Roche Diagnostic Corp.). And amount of protein was determined using a dye-binding microassay. Equal amounts of protein were electrophoresed on 10% SDS poly-acrylamide gels followed by electrotransfer onto a polyinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membranes were blocked for 1hr with TBS-T (10mM Tris-HCl (pH 7.4), 150 mM NaCl and 0.1% Tween-20) containing 5 % skim milk and then incubated at 4° C with using the following anti-

bodies: mouse anti-PSR(H7) and mouse anti-$\beta$actin from Santa Cruz Biotech-nology. and developed using an enhanced chemiluminescence detection system (ECL; Millipore, Buckinghamshire, UK).

## 8. Transwell Migration assay

In vitro cell migration assays were performed in a 24well Transwell plate with 8㎛ Polycarbonate Membrane Tissue Culture Treated, Polystyrene(Costar, USA) separating the lower and upper culture chambers. $4 \times 10^4$ cells were plated in serum free media in upper chamber. Complete media were added to the bottom wells. Non-migrated cells were removed from the upper face of the filters using cotton swabs, and migrated cells in the lower face of the filters were fixed with Hemacolor Solution 1, Fixing solution (Millipore, USA) and stained with Hema-color Solution 2 and 3 (Millipore, USA).

## 9. IncuCyte Migration assay

Plate cells on top of thin-layer matrix, and allow them to adhere for 24

hours. next, create wound area using Essen 96-well WoundMarker™ in just seconds. Layer collagen gel on top of cells to create 3D matrix. And then, the IncuCyte automatically acquires images for the entire duration of the assay. After assay is completed, the IncuCyte's integrated software provides the means to quantify cell migration.

## 10. Statistical analysis

Each experiment was performed at least three times. The data in all experiments are represented as mean ± standard deviation (S.D.). Statistical comparisons were carried out using an unpaired t-test. P-values<0.05 were considered statistically significant.

# RESULT

## 1.The expression pattern of JMJD6 mRNA variants breast cancer.

JMJD6 is known to poor prognosis in breast cancer.[23] We investigated whether breast cancer patient dataset in The Cancer Genome Atlas (TCGA) contain truncated mRNAs. The RNA-seq data from 106 solid tumor samples and matching normal tissue samples were used. Expression of truncated mRNAs annotated in RefSeq was examined. We found that the distribution of truncated JMJD6 mRNA in cancer tissues was higher than normal tissues from breast cancer patients (Fig 1A). The expression of truncated JMJD6, named in this study as JMJD6SF, was highly expressed in tumor samples compared to matching normal tissues. Notably, the expression of full-length JMJD6 mRNA, named in this study as JMJD6LF, was slightly decreased in tumor samples compared to normal tissues (Figure 1). These results demonstrated that cancer-elevated truncated JMJD6 mRNA is co-related with clinical outcomes in breast cancer.

## 2.The expression of JMJD6 transcript variants in human breast cancer cell lines.

Next, we asked whether cancer-elevated truncated JMJD6 mRNA was expressed in the breast cancer cell line. To understand whether two transcript variants of JMJD6 have different roles, we refer to the database of nucleotide sequences in NCBI to illustrate structural domain. (Figure 2A) When we identified the sequence, compared to JMJD6 LF (NM_001081461), we found that there was a defect in the C-terminal part (shaded areas) of JMJD6 SF (NM_015167). We estimated that the role of JMJD6 LF and JMJD6 SF, which are transcript variants of JMJD6, are different due to these depletion parts of JMJD6 SF.

We performed Real-time PCR to examine the expression level of JMJD6 LF and JMJD6 SF by line of breast cancer cell lines. Compared with MCF10A, which is a normal breast cell, the expression levels of JMJD6 SF were increased in most breast cancer cell lines compared to JMJD6 LF.

Next, RT-PCR was performed on MCF-7 cells by preparing primers to measure the relative amount in the breast cancer cells. As a result, it was quantified using Image J software that the amount of SF was twice as much as that of LF. (Figure 2C, left panel) When real-time PCR was performed to approach this more quantitatively, the expression level of SF was relatively higher than LF. (Figure 2C, right panel) We hypothesized that SF would contribute more to the progression of cancer because of the relatively higher number of SF present in the breast cancer cell MCF-7.

### 3. JMJD6 transcription variants exhibit a different phenotype in breast cancer cell.

For in vitro experiments, plasmid DNA of JMJD6 transcript variant form was purchased. MCF7 cells were transiently transfected with JMJD6 LF and JMJD6 SF expressed vector, and after 48hr harvested. Indicated total lysates were western blot analysis using a turbo-GFP antibody. (Figure 3A) Plasmid DNA was transfected into MCF-7 cells and then subjected to western blot analysis. MCF-7 cells were transfected and seeded 48 hours later. Stable cells were pre-pared by treating 500 ㎍ of G418 for 2 weeks and pooling colony. (Figure 3B) Observing the morphology through a microscope after preparing a stable cell, the phenotype of JMJD6 transcript variants overexpressed MCF-7 cells was changed. (Figure 3C)

MSCs have recently been described to localize within breast carcinomas where the stem cells integrate into tumor-associated stromal tissues whereby

the MSCs promote breast cancer cell invasion and metastasis.[24] Mesenchymal cells are unique spindle-shaped cells. The expression of JMJD6 SF profoundly changed cell morphology to a mesenchymal-like phenotype and induced a loss of contact inhibition compared to JMJD6 LF cells that have an epithelial-like appearance. The ability of tumor cells to form a metastatic tumor is primarily determined by the cell's ability to change and recognize its cellular morphology, and to degrade the extracellular matrix (ECM). Based on this result, we can deduce that SF migration ability will be higher than LF.

### 4. Overexpression of JMJD6 SF enhances migration ability in breast cancer cells.

In the phenotypes data (Figure 3C), the LF and SF of JMJD6 overexpressed in MCF-7 cells resulted in different phenotypes, and in particular, SF showed an EMT-like phenotype and we speculated that the ability to migration in SF would be higher.

Cell mobility is important to tumor severity. Therefore, to confirm this data, we conducted an IncuCyte assay to compare the migration ability of LF and SF. Cells were seeded on a 96-well essen lamgelock plate and 24 hours later, a 96-pin woundmaker was used to generate accurate wounds in all wells on the cells. During plates were placed in a 37℃ incubator, IncuCyte software scans the plate every hour for migration analysis. Analyzing the scanned images at 0 hours, 24 hours, and 43 hours, the yellow line was the part that originally caused the wound. The red line was the area where the wound healed and migrated. It can

be seen that JMJD6 SF has more wound healing than LF and Serum free MCF-7. This means that JMJD6 SF has the most migration effect. (Figure 4A)

In order to measure migration capability and confirm previous data in a more general way, we conducted a Transwell migration experiment. In the Transwell migration assay, the JMJD6 SF migration rate increased from 100 to 181.7 % compared with MCF7. (Figure 5B) But JMJD6 LF migration rate was no effect. Therefore, Transwell migration data also revealed that JMJD6 SF has much bet-ter migration capability than JMJD6 LF. (Figure 5A)

### 5. Knockdown of JMJD6 SF suppresses migration ability in breast cancer cells.

In the preceding data, we examined the effect of JMJD6 overexpression on breast cancer cells. To determine whether the same pattern appears when knock down JMJD6, we designed the siRNAs. The knockdown efficiency of the siRNAs was detected by RT-PCR, Real-time PCR and western blot analysis. (Figure 6A and B) It was confirmed that JMJD6 SF was transiently reduced upon si transfection. However, when siRNA against JMJD6 LF was transiently si transfected, no decrease was observed. This was presumably due to the fact that the amount of SF present in the breast cancer cell is relatively large compared to LF. (Figure 2C)

To confirm the previous overexpression migration experiment, JMJD6 LF and SF in MCF7 cells Transwell migration assay was performed with transient knockdown cells. As expected, the migration in depleted JMJD6 LF cells increased compared to MCF-7 cells. Conversely, when depleted JMJD6 SF cells

transfected, migration decreased. (Figure 6C and D) We concluded that JMJD6 SF promotes mobility in breast cancer cells.

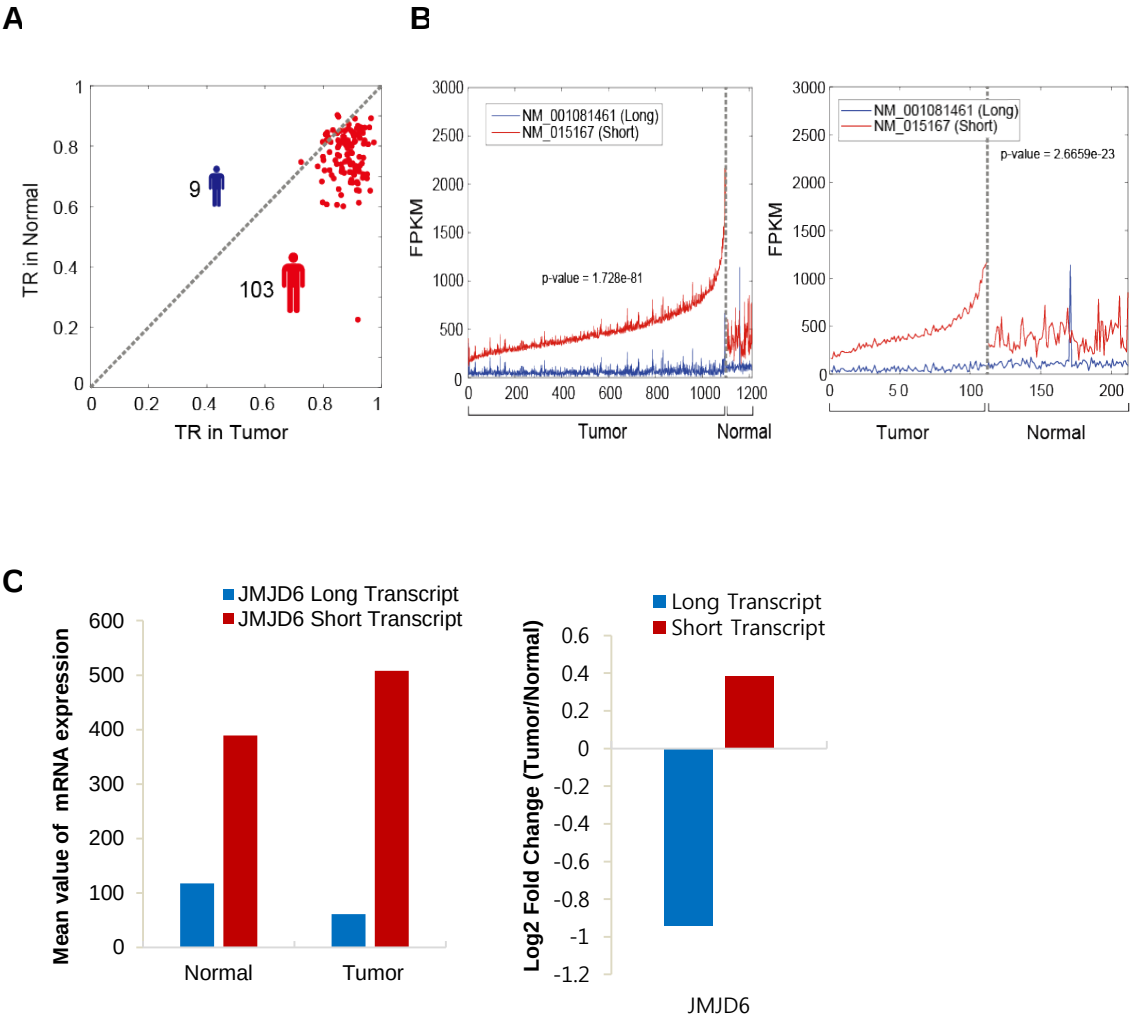
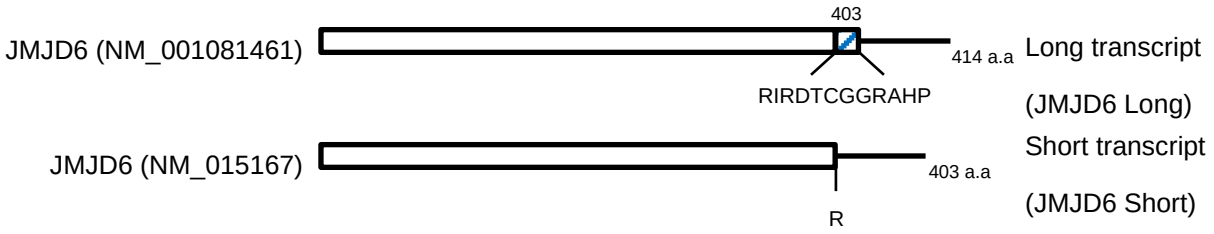


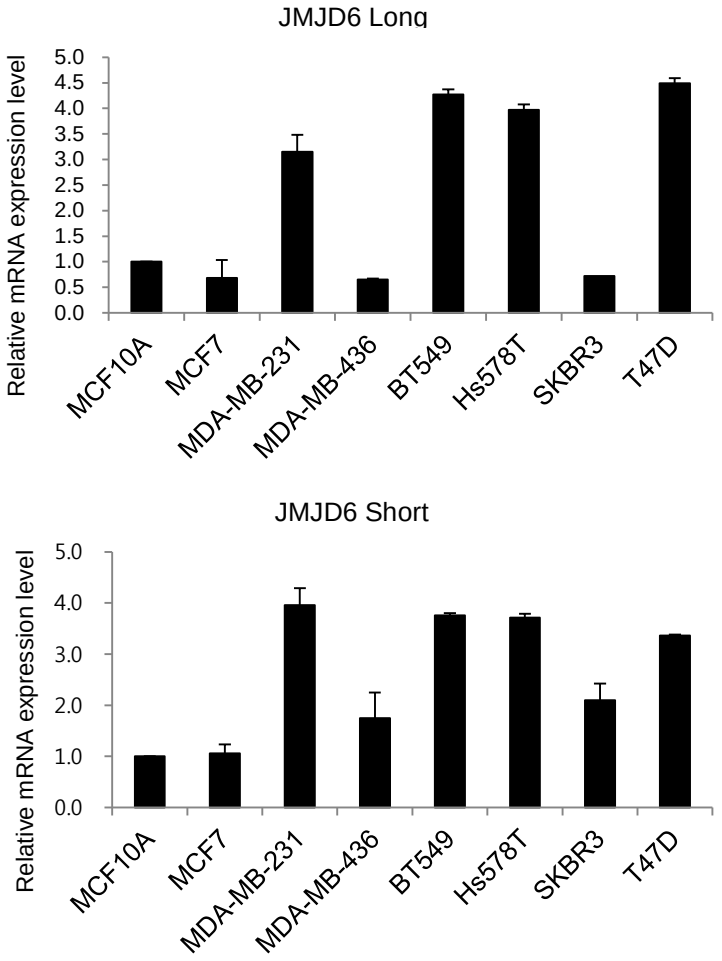
Figure 2. JMJD6 transcript variants expression levels in breast cancer (BRCA) patients.

(A) Scatter plot of JMJD6 mRNA expression in normal and tumor from BRCA patients. (B) Analysis of JMJD6 transcript variants mRNA expression in TCGA cohort (TCGA cohort n=1,116, $p<0.001$, left panel). Re-analysis of JMJD6 transcript variants mRNA expression in 100 paired BRCA patients and adjacent normal tissue from TCGA cohort (TCGA cohort n=100, $p<0.001$, right panel). (C) Expression of JMJD6 transcript variants mRNA expression in normal and tumor from BRCA patients. Graph represented the mean of JMJD6 transcript variants mRNA expression in TCGA cohort.

**A**




**B**



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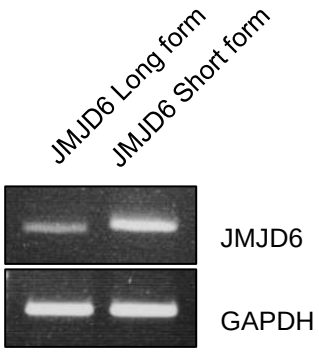
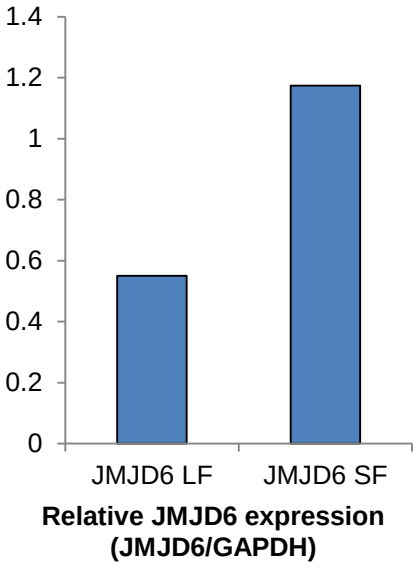


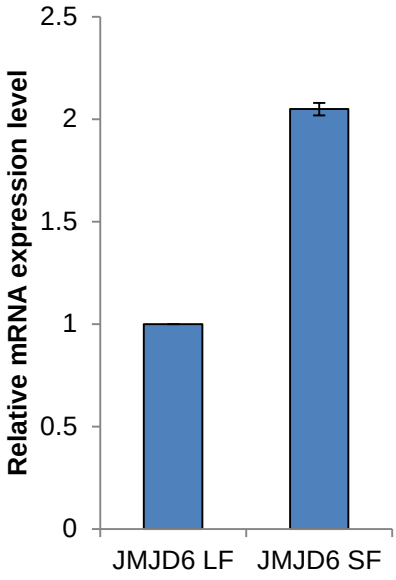



**Figure 3. JMJD6 transcript variants expression levels in breast cancer cell lines.**

(A) Schematic alignments of JMJD6 transcript variants. The JMJD6's two isoforms generated through alternative splicing. These two protein isoforms are called the JMJD6 long form and JMJD6 short form. These two products differ in their C-terminal part. (B) Expression of JMJD6 in breast cancer cell line was confirmed by Real time PCR. MCF-10A is a normal breast cells. (C) Western blot analysis, RT-PCR and Real-time PCR experiments were used to compare the expression of JMJD6 transcript variants LF and SF in MCF-7.

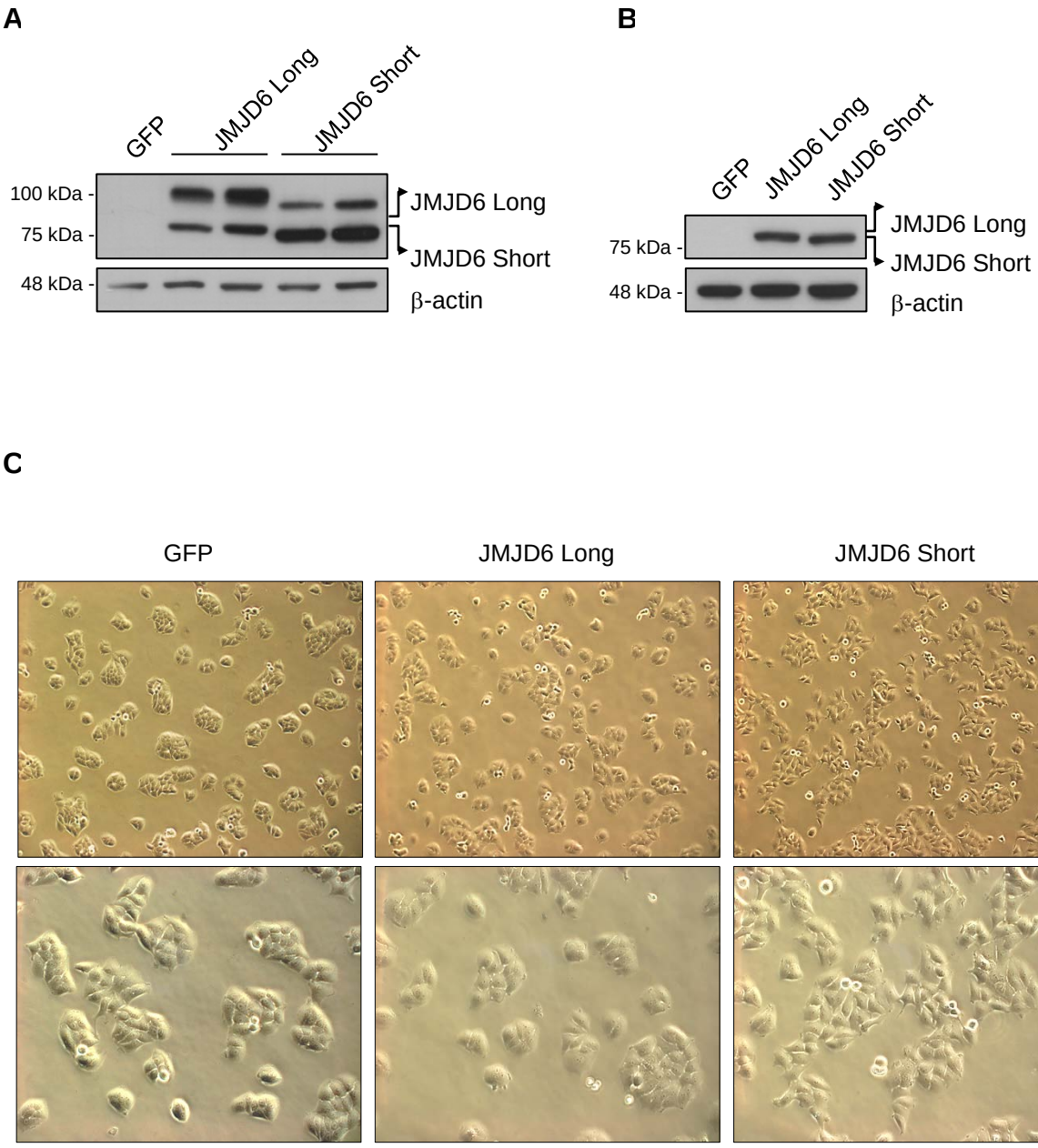
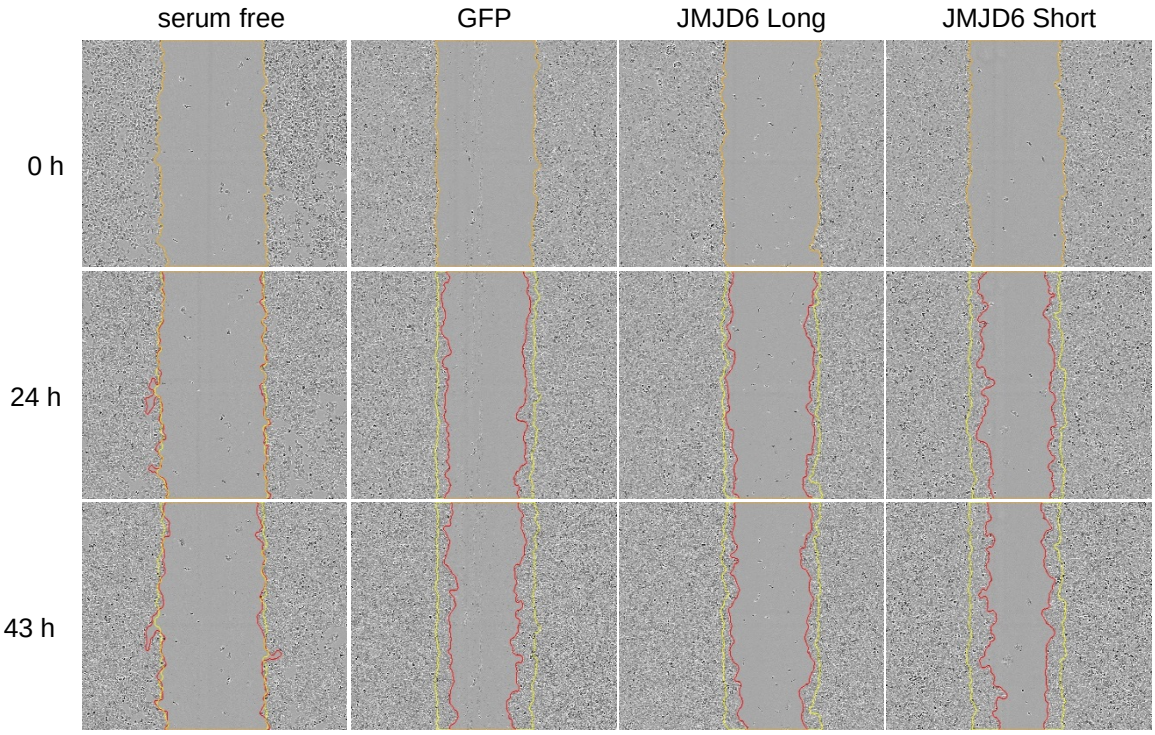


Figure 4. Establishment of JMJD6 transcript variants over-expressed MCF-7 cells.

To determination expression of JMJD6 in breast cancer cell, (A) Plasmid DNA bought from Origene. To assess the exact role of JMJD6 on MCF-7 cells, we generated MCF-7 cells lines stably overexpressed JMJD6 LF and SF. (B) Selected with 500㎍/㎖ G148. After 2 weeks, cells were pooling and GFP expression was confirmed by western blot analysis. Western blot analysis showed the JMJD6 expression in MCF-7 cells and compared with the control vector expressing cells. β-actin was used as a loading control. (C)In vitro characteristics expressing JMJD6 overexpressed in MCF-7 cells. Pictures were taken at 40X and 200X using light microscopy.

**A**

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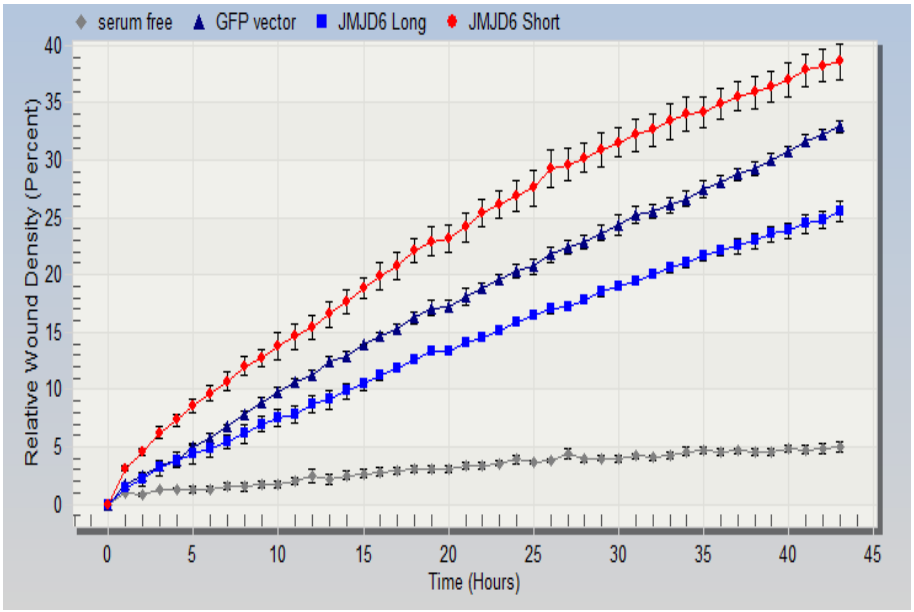
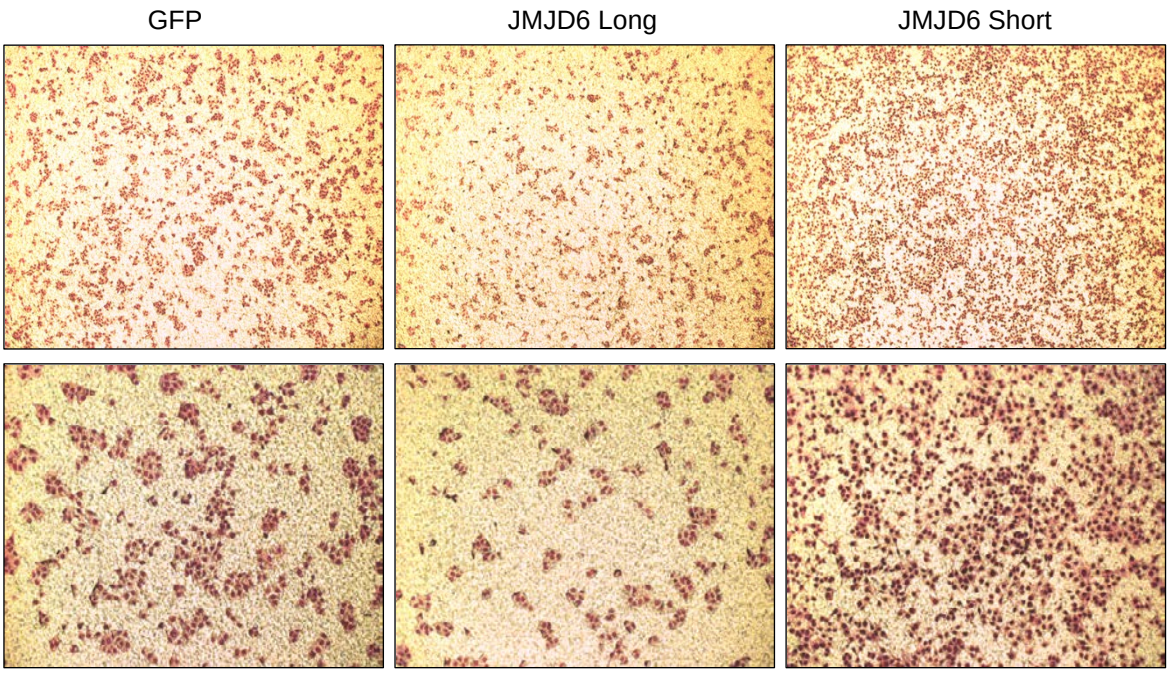


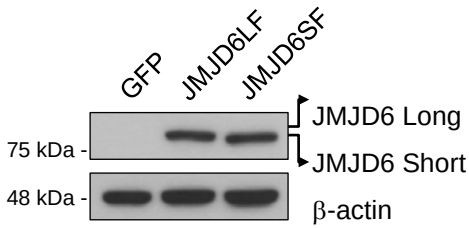
Figure 5. Effect of cell migration of JMJD6 transcript variants over-expressed MCF-7 cells using IncuCyte.

(A) The yellow line is the part that originally caused the wound. The red line is the area where the wound healed and migrated. As a result, compared to the control and LF, the most migrated area of SF is the most. (B) The migrated area was quantified and plotted.

**A**




**B**



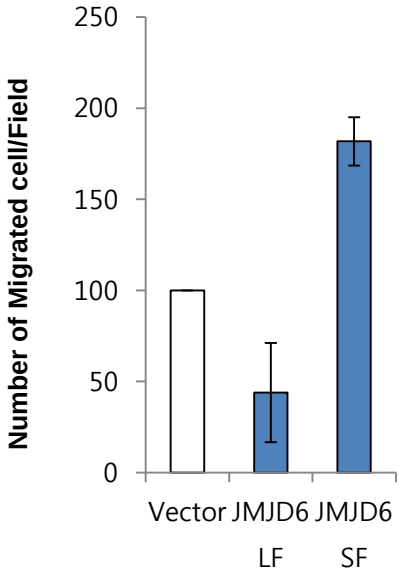
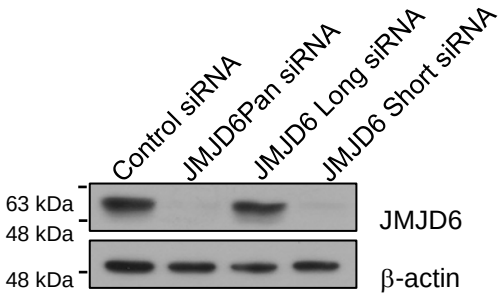



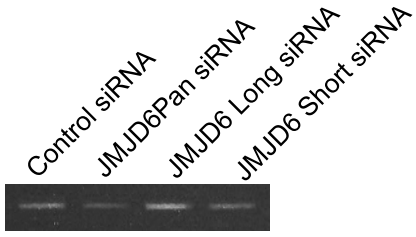
Figure 6. Effect of cell migration of JMJD6 transcript variants over-expressed MCF-7 cells using Transwell.

(A) Cell migration assay were performed using the Transwell system. Data represent the mean cell numbers 5 fields. (B) Western blot analysis showed the increase of JMJD6 expression in JMJD6 overexpressing MCF-7 cells, compared to control vector expressing cells. $\beta$-actin is loading control. (Left panel) The percent of migration was determined. (Right panel)

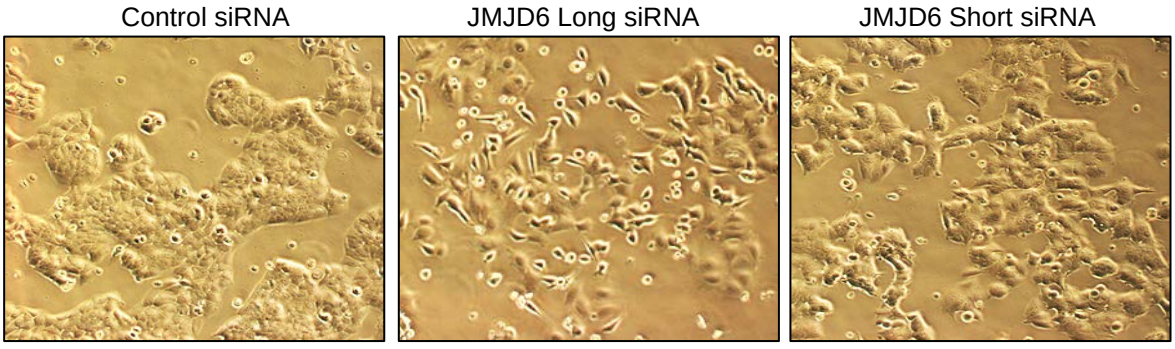
**A**



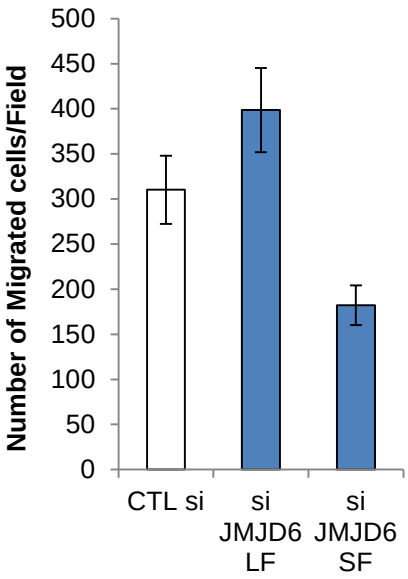

**B**




**C**



D



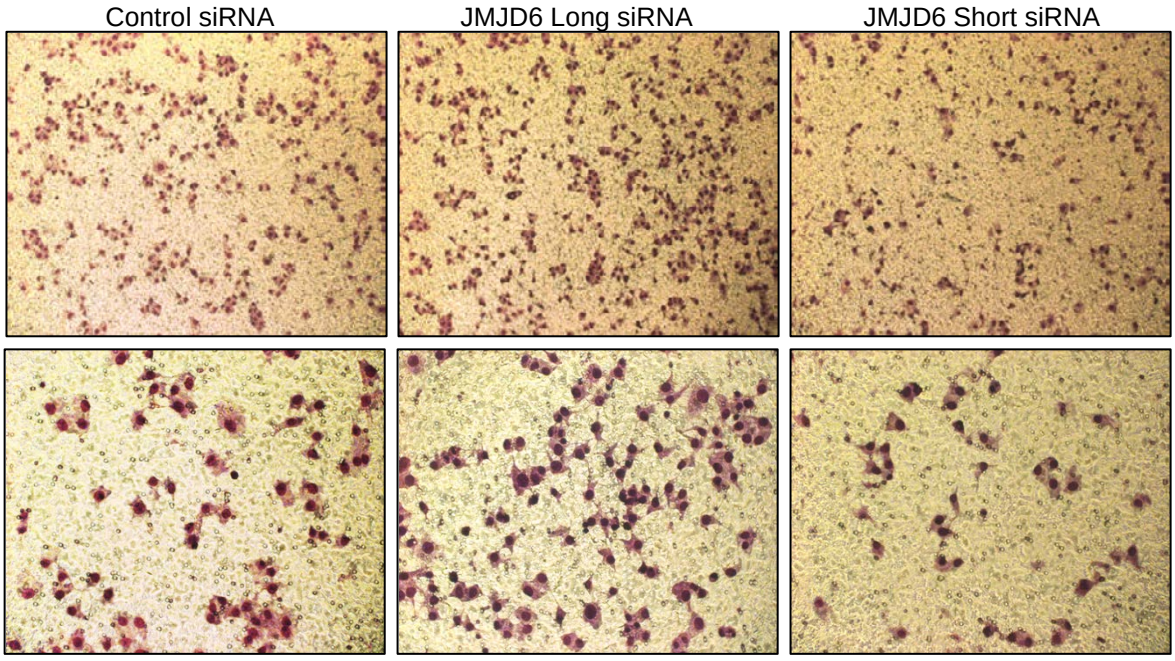



Figure 7. Effect of cell migration of JMJD6 transcript variants knockdown MCF-7 cells.

(A)MCF-7 cells were transfected with siRNA targeting JMJD6 with Lipofectamin RNAimax reagent in opti-MEM for 48hours. Expression of JMJD6 was confirmed by western blot analysis using JMJD6 antibody. $\beta$-actin used as loading control. (B)siRNA effect confirmed by RTPCR. (C)It is the phenotype that appears when MCF-7 cells are siRNA transfected with JMJD6. Pictures were taken at 40X and 20X using light microscopy. (D) Cell migration assay were performed using the Transwell system. Data represent the mean cell num-bers 5 fields.

# DISCUSSION

Previous studies have shown that JMJD6 is known to modulate estrogen non-genomic signaling by demethylating ER$\alpha$ [25]. Also JMJD6 has been reported as a lysyl-5-hydroxylase in the splicing factor U2AF65 [20] and histones H3 and H4 [26], indicating that JMJD6 exhibits dual enzyme properties. JMJD6 also regulates gene expression by regulating RNA splicing, suggesting that JMJD6 is a multifaceted epigenetic regulator. Clinically, the overexpression of JMJD6 is closely associated with poor prognosis in a variety of human cancers, including lung and breast. JMJD6 has been reported to promote cancer cell migration, invasion and angiogenic sprouting, two phenotypes of which are well known for cancer stem cell (CSC; which was known as cancer-causing cell) characteristics.

In BRCA patients, we found that JMJD6 expresses much more on mRNA in tumor tissues than normal, suggesting that the JMJD6 will cause a lot of impact

in breast cancer. Analysis of JMJD6 transcript mRNA in normal tissue from adjacent to 100 pairs of BRCA patients in TCGA cohort shows that the high expression rate of JMJD6 SF in the tumor. Based on the fact that JMJD6 is reported to be a poor prognosis in the breast cancer [23], and that expression of JMJD6 is highly expressed in BRCA patients, especially short transcript of JMJD6, we investigated the role of JMJD6 transcript variants, JMJD6 LF and JMJD6 SF by alternative splicing in breast cancer cell. RT-PCR and Real-time PCR experiments confirmed that JMJD6 SF showed high expression levels in MCF-7 breast cancer cell. To further determine the role of JMJD6 in cancer cell function, we established the JMJD6 LF and SF overexpressed MCF-7 cells, respectively. Then, JMJD6 SF was phenotype with cancer cell characteristics was observed. Therefore, we carried out the Incucyte assay and the Transwell migration assay experiment because we thought that JMJD6 SF would be cancer migration ability. As expected, JMJD6 SF confirmed the high migration capability.

The result was also obtained when depletion of JMJD6 to confirm the previous data. Therefore, our data suggest that JMJD6 SF may contribute to the tumorigenicity of breast cancer cells.

In conclusion, by using a combination of other in vitro approaches, we have demonstrated that JMJD6 SF possesses tumor characteristics. And, the study about JMJD6 expression in the cohort of breast tumors showed that JMJD6 SF could be an indicator of a poor prognosis. Further studies are required to clarify the role of JMJD6 in breast cancer and its precise mechanism.

# ABSTRACT

## The functional role of alternative splicing variants in cancer progression

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Alternative pre-mRNA processing is an important regulator of gene expression because it produces sufficient copies of the use of genetic information, in which exons remain and introns are removed. Thus, alternative splicing is an important mechanism for gene regulation and genetic diversity.

Jumonji Domain Containing6 (JMJD6) is a histone arginine dimethylase that allows the dynamic regulation of transcription by preferentially removing the methyl group form the arginine 3 of histone 4 (H4R3me2) and the demethylated

arginine 2 of histone 3 (H3R2me2). JMJD6 also regulates gene expression by regulating RNA splicing, suggesting that JMJD6 is a multifaceted epigenetic regulator. Clinically, overexpression of JMJD6 is closely associated with poor prognosis in a variety of human cancers, including lung and breast. JMJD6 has been reported to promote cancer cell migration, invasion and angiogenic sprouting, two phenotypes of which are well known for cancer stem cell (CSC; known as cancer-causing cells) characteristics.

Recent studies have reported that alternative splicing are closely associated with various diseases, including cancer. However, its functional consequence of cancer progression remains unknown. We have been discovered the cancer-related JMJD6 transcript variants through cancer database analysis.

In this study, we identify two distinct protein of JMJD6 that differential expression changes in cancer tissues: full-length JMJD6 mRNA expression decreased in cancer, whereas truncated JMJD6 mRNA expression increased in

cancer, compared to normal tissue. We suggest that truncated JMJD6 mRNA by alternative splicing is mainly associated with cancer.

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