Analyses of molecular and cellular mechanisms of hypothetical protein FAM188B function in gastrointestinal cancer

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Department of Medical Science

The Graduate School, Yonsei University

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Directed by Professor Hyoung-Pyo Kim

The Doctoral Dissertation
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Eun-Seok Choi

June 2018

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June 2018

ACKNOWLEDGEMENTS

처음 박사학위과정을 시작하면서 이 기간 동안 최대한 많이 공부하고 여러 가지 실험기술도 배우자는 생각을 했었습니다. 하지만 시간이 지나며 배운다기보다는 스스로 해결해야 한다는 생각이 더욱 커졌습니다. 아마도 박사학위 과정이 무조건 배우는 과정이 아닌 탓이었기 때문이라고 생각합니다. 도중에 여러 사람들을 만나고 또 먼저 박사학위를 마친 분들의 글을 보면서 항상 나는 이 공부를 하면서 무엇을 성취하려고 하는지, 박사학위를 받은 분들은 어떤 자격을 갖추면 무사히 졸업을 할 수 있었는지 고민을 하였습니다. 이러한 과정을 무사히 잘 마칠 수 있도록 조언을 아끼지 않으며 지도해 주신 고성호 박사님, 김형표 교수님 두 분께 가장 감사하다는 말씀을 드리고 싶습니다. 또한 논문 심사를 진행하며 올바른 논문을 적도록 많은 도움을 주신 전경희 교수님, 김명희 교수님, 이상길 교수님께도 감사의 말씀을 드립니다.

박사과정으로의 진학결정, 그리고 연구 논문이 하나하나 나올 때 도움을 주신 김경태 박사님, 김용연 박사님, 최용두 박사님, 장현철 박사님, 이상진 박사님, 성지영 박사님께 감사의 말씀을 드립니다. 또한 같이 생활하며 연구 분야와 관심사는 다르지만 진심 어린 충고와 조언을 해주신 연구원 선생님들께 항상 감사의 마음을 가지고 있

었지만 표현을 하지 못한 점 양해 드리고, 이 지면을 빌어 감사의 말을 전합니다. 미처 다 적지는 못하였지만 지난 학위과정 동안 만난 모든 인연에 고맙다고 생각하고 있습니다.

언제나 저를 믿어주신 사랑하는 부모님, 그리고 나의 동생에게도 감사의 말을 전합니다.

국립암센터-연세대학교 학연협동과정으로 입학하게 해 준 두 기관도 저의 졸업에서는 무척이나 고마운 곳입니다. 아직 부족한 점이 많지만 졸업을 하고서도 계속해서 과학에 관심을 가지며 도움이 되는 사람이 되려고 합니다. 앞으로는 학교에서 배우는 것이 아닌 홀로 문제에 대해 고민하고 해결해야 하겠지만 지금껏 배우고 익힌 모든 것을 활용하여 잘 헤쳐 나가도록 하겠습니다.

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ABSTRACT

Analyses of molecular and cellular mechanisms of hypothetical protein FAM188B function in gastrointestinal cancer

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It has been previously reported that FAM188B has significant differential exon usage in cancers (NCBI GEO GSE30727), but the expression and function of FAM188B are not well characterized. Here, I explored the functions of FAM188B by a knockdown strategy, using siRNAs specific for FAM188B in gastric and colon cancer cell lines. *FAM188B* is a novel gene encoding a protein that is evolutionarily conserved among mammals. Its mRNA has been found to be highly expressed in most solid tumors, including gastric and colorectal cancer. FAM188B knockdown induced apoptosis in cancer cell lines, and simultaneous treatment of anticancer drugs and siFAM188B had a synergistic

effect on reducing cell viability in gastric cancer. To identify the underlying mechanism, siFAM188B was transfected into colon cancer cell lines. Interestingly, siFAM188B treatment induced the upregulation and activation of p53, and consequently increased the p53-regulated pro-apoptotic proteins PUMA and BAX. Proteomic analysis of FAM188B immunocomplexes revealed p53 and USP7 as putative FAM188B-interacting proteins. Deletion of the putative USP7-binding motif in FAM188B reduced the interaction of FAM188B with USP7. It is noteworthy that FAM188B knockdown resulted in a decrease in overall ubiquitination of p53 immunocomplexes, as well as p53 ubiquitination, because USP7 is involved in p53 deubiquitination and FAM188B has recently been proposed as a putative deubiquitinase. FAM188B knockdown inhibited both colony formation and anchorage-independent growth *in vitro*. In addition, *FAM188B* knockdown by siRNA reduced tumor growth in xenografted mice, with an increase in p53 expression. Taken together, our data suggest that FAM188B has a putative oncogenic function, controlling p53 stability to promote cell growth. Therefore, control of FAM188B could be a possible target to inhibit tumor growth.

Key words: FAM188B, p53, hypothetical protein, cancer, deubiquitinase USP7

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I. INTRODUCTION

1. Stomach cancer and colon cancer

Stomach cancer is the third leading cause of cancer death in both sexes worldwide (723,000 deaths, 8.8% of the total). In particular, the highest estimated mortality rates are in Eastern Asia (24 per 100,000 in men, 9.8 per 100,000 in women).[1] Thus, it is necessary to develop a therapeutic strategy to overcome the high mortality rates caused by stomach cancer. Stomach cancer is composed of heterogeneous cell populations that manifest malignancy by aberrantly regulating cell proliferation, differentiation, angiogenesis, migration, and metastasis,[2] and its carcinogenic process is complex.[3,4]

Colorectal cancer (CRC) is the third most prevalent cancer worldwide and is a major contributor to cancer mortality.[5] CRC is heterogeneous disease, biologically classified into three major groups according to their molecular characteristics. The first is the chromosomal instable group, which accumulates mutations in specific oncogenes and tumor suppressor genes. The second class is the microsatellite instability (MSI) group, which leads to genetic hyper mutation, and the third is distinguished by CpG island methylation.[6] In addition, large-scale genomic studies have been conducted to advance our understanding of CRC at a molecular level, including The Cancer Genome Atlas analysis of 276 colon cancer patients.[7] Many critical pathways contribute to the development of CRC, including APC, WNT, RAS-MAPK, PI3K, TGF-β, TP53, and DNA mismatch repair.[7] However, despite these efforts, there is still lack of detailed characterization for low to intermediate frequency mutations or novel candidates. Therefore, it is necessary to continue to search for novel genes related to carcinogenesis.

2. Hypothetical protein FAM188B

Substantial proportions of genes (59%) in the human genome are reported as 'hypothetical' and are annotated as being of 'unknown function'.[8] Hypothetical proteins are predicted from nucleic acid sequences and their existence has not been experimentally proven. Another feature of the hypothetical protein is that it has low identity compared to known proteins.[9] However, despite their

hypothetical status, which can be an obstacle to investigations of their expression patterns and potential functions in cellular pathways, such genes are often expressed to varying degrees in disease and are therefore biomedical relevant.[10] Thus, excluding 'unknown' or 'hypothetical' genes from analyses of candidate targets removes the opportunity to explore unprecedented molecular mechanisms that may be involved in clinically significant pathological dysfunctions. Recently, a hypothetical protein, FAM63A, was characterized to interact with ubiquitin and identified as a new DUB family member, and the analysis of evolutionarily conservation among human genomes identified several homologues including FAM63B, FAM188A, and FAM188B. The comparison of protein sequences showed these proteins shared a conserved region, a catalytic domain for the hydrolysis of ubiquitin at the carboxyl terminal of proteins. They were named MINDY (motif interacting with Ub containing novel DUB family) lysine 48 deubiquitinase.[11]

3. Apoptosis and cancer

Programmed cell death inhibits the development of cancer naturally through apoptosis of abnormal cells, but cancer develops when this mechanism is disrupted.[12] Typically, when chromosomal abnormality occurs, the expression of tumor suppressor P53 is increased, leading to apoptosis of the cells.[13] Regulation of p53 is controlled by various post-translational modifications. The ubiquitin-proteasome system (UPS) is the main pathway for controlling protein

integrity, and is central to the regulation of many cellular functions, notably including cell survival and death.[14, 15] Ubiquitination is a remarkably complex, specific, three-enzyme (E1-E2-E3) cascade that utilizes two E1, ten E2, and hundreds of E3 ubiquitin ligases.[16] Deubiquitinases (DUB, ubiquitin isopeptidase) are UPS components that catalyze removal of an ubiquitin moiety from poly-ubiquitin chains,[14] the human genome encodes 98 DUB genes classified into 6 families.[17] Thus, the dynamic and combinatorial interactions between ubiquitination and deubiquitination set the threshold for apoptotic signaling.[18] For example, the E3 ubiquitin ligase MDM2 ubiquitinates the tumor suppressor p53, and DUBs, such as ubiquitin-specific proteases USP2a, USP7, USP10, USP22, and USP42, are involved in regulating the stability of p53 and MDM2 by removing ubiquitin moieties.[14, 19-21] However, what determines whether p53 or MDM2 is the primary USP substrate is not known.[18, 22]

II. MATERIALS AND METHODS

1. Datasets and extraction of differentially expressed genes

The mRNA expression profiling for cancer cell lines was obtained from Cancer Cell Line Encyclopedia (CCLE) database (https://portals.broadinstitute.org/ccle).[23] Gene expression datasets for colorectal cancers (GSE20842[24] and GSE20916[25] were retrieved from NCBI Gene Expression Omnibus and TCGA Colorectal 2 (215 colorectal adenocarcinoma and 22 paired normal colorectal tissue samples were analyzed. This dataset is a combination of Colon Adenocarcinoma [COAD] and Rectum Adenocarcinoma [READ] data from the TCGA data portal and consists of Level 2 (processed) data. Corresponding DNA copy number data is available in TCGA Colorectal 2.)) These datasets were used for the analysis of the up-regulated genes of colorectal cancer in Oncomine (https://www.oncomine.org/).[26] Differentially expressed genes were extracted by selecting discrimination criteria for >2x of expression difference with p-value < 0.001 between normal tissues and colorectal adenocarcinoma. PANTHER Gene Ontology tool was used to classify the overlapping genes and I focused on the “unclassified” 155 genes which are most frequently classified and are not belonged to any three classification criteria (biological process, molecular function and cellular component).[27]

2. Drug resistance test

Cells, plated in 6-well culture plates ($8x10^4$ cells/well), were transfected with 5 nM siFAM188B or NC siRNA using Lipofectamine RNAiMAX (Thermo Fisher Scientific, San Jose, CA, USA). All drugs (etoposide and cisplatin) were treated at various ranges of concentrations (0.5~4.0 μM and 2.5~40.0 μM, respectively) 24 hr after siRNA transfection. Cytotoxicity was measured at 48 hr and 72 hr after drug treatment by tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

3. Short hairpin RNA (shRNA) treated stable cell lines

For lentivirus production, HEK293T cells were seeded at a density of 3.5×10^6 cells per 100 mm dish. Five plates for each constructs were prepared and incubate at 5% CO_2, 37°C incubator for 24 hr. The mixture of plasmids (FAM188B-targeting shRNA plasmids, pSPAX2, and pMD2.G) was transfected into the prepared cells next day. Supernatant of HEK293T cells was harvested after 48 hr and concentrated with Lenti-X concentrator (Clonetech) at 4°C for 16 hr. Concentrated viruses were infected into prepared HCT-116 cells in 6-well plates (3.0×10^5 cells/well) with Polybrene (Sigma-Aldrich). Media was replaced after 6 hr incubation to remove the remained viruses. shFAM188B infected cells were classified as GFP signal and collected by flow cytometry.

FAM188B-targeting shRNA sequences: shFAM188B#1 (5'-CTT TGG AAA TAC GGC TAA CAA-3') and shFAM188B#2 (5'-CAG ATA CTT TCT GGA

TCA CTT-3')

4. Analysis of the FAM188B sequence conservation

Amino acid sequence of *Larimichthys crocea,*[28] *Nothobranchius kadleci, Nothobranchius rachovii, Aphyosemion striatum, Fundulus heteroclitus, Xenopus tropicalis,*[29] *Alligator mississippiensis,*[30] *Mus musculus,*[31] *Bos taurus, Callithrix jacchus,*[32] *Macaca fascicularis,*[33] *Macaca mulatta,*[33] *Pongo abelii, Pan troglodytes,* and *Homo sapiens*[34] were obtained from GenBank. Sequences were aligned using Clustal Omega software[35] and draw the phylogram.

5. Cell culture and human tissue samples

The human cell lines used in these studies (HCT-116, SW620, HT-29, AGS, SNU-638, A549, U87, JIMT1, MDA-MB-231, HeLa, HEK293 and HDF) were obtained from American Type Culture Collection (Manassas, VA, USA) or Korean Cell Line Bank (Seoul, Korea). All the cells were cultured with designated media (Corning, Manassas, VA, USA) supplemented with 10% fetal bovine serum (Corning) and 1x Penicillin Streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C containing 5% CO_2.

Fresh human stomach tissue samples were obtained from patients undergoing surgery and were stored in RNA Later solution (Qiagen, Hilden, Germany) for total RNA extraction or in liquid nitrogen for protein extraction. All patients provided informed consent prior to collection of tissues, and the

study was approved by the Institutional Review Board of National Cancer Center, Korea (NCCNCS13732).

6. Reverse transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Waltham, MA, USA) according to the manufacturer's protocol and was purified using an RNeasy column with RNase-free DNaseI treatment (Qiagen, Hilden, Germany). First-strand cDNA was synthesized from 1 μg of total RNA using a Transcriptor cDNA Synthesis kit (Roche, Basel, Switzerland) and poly-d(T)18-21 primers.

Primers and probes for RT-PCR and qRT-PCR that spanned two consecutive exons were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/). qRT-PCR reactions were performed using a LC480 real-time PCR machine (Roche). *FAM188B* targeting probe sequences: [FAM]-TGT TTG AAG GAG ATA GCA AAG CCG ACT GTG-[TAMRA]

Primer sequences are listed in Table 1.

Table 1. Primer sequences for PCR reactions

Target name	Forward primer (5' – 3') Reverse primer (5' – 3')	Annealing temp. (°C)
FAM188B	GAG TCC TGG CAG CTG TCC AA GCA TCT GAA GGC TGC AGT CC	54
ACTB	CAT CGA GCA CGG CAT CGT CA TAG CAC AGC CTG GAT AGC AAC	58
BAX promoter_p53RE	TTG GGC TCA CAA GTT AGA GAC AAG CCT GGA TCT AGC AAT ATA GCC CAC	60
PUMA promoter_p53RE	TCA GTG TGT GTG TCC GAC TGT C GGC AGG GCC TAG CCC A	60

7. Plasmid constructs

FAM188B was cloned into pGEM-T easy, pFLAG-CMV2, pAcGFP-C1, pAcGFP-N3, and pcDNA3.1 vector. Ubiquitin tagged with HA and p53 wild type constructs were cloned into pcDNA3.1 vector. pCl-neo-FLAG-HAUSP (FLAG-tagged USP7) was obtained from Addgene. *FAM188B*-targeting shRNA sequences were cloned into pHRST vector. pSPAX2, and pMD2.G were used for lentivirus propagation.

8. FAM188B antibody generation

For the detection of FAM188B, I generate polyclonal antiserum by immunization with hemocyanin-conjugated peptides with sequences predicted

to be antigenic as well as specific for the FAM188B protein C-terminus (722~738 amino acid residues: TISEDTDNDLVPPLELC - 17mer) (AbFrontier, Seoul, Korea).

9. Western blot analysis

Harvested cells were lysed using M-PER Mammalian Protein Extraction Reagent (Thermo Fisher scientific) with protease inhibitor cocktail (Roche). Protein separation was performed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Billerica, MA, USA). Membranes were blocked with 5% skim milk and incubated following antibodies: FAM188B (F374C), GAPDH, GFP (JL-8), p53 (DO-1), p53 (FL393), p-p53 (Ser15), p21, BAX, PUMA, USP7, α-tubulin, HA, ubiquitin, FLAG-M2, PARP, Caspase-3

10. Small interfering RNA transfection

FAM188B expression was silenced in HCT-116 colon cancer cells by transfecting with *FAM188B*-specific siFAM188B (Qiagen) targeting the sequence 5'-CTG ACC ATT GAC ACC ACC CAA-3', Allstar negative control (NC) siRNA (Qiagen) was used as a negative control. Cells, plated in 6-well culture plates (8×10^4 cells/well), were transfected with 5 nM siFAM188B or NC siRNA using Lipofectamine RNAiMAX (Thermo Fisher Scientific) according to the manufacturer's protocol.

11. DNA fragmentation assay

Cells were seeded on coverslips and incubated at 37°C containing 5% CO_2 incubator for overnight. After 4% paraformaldehyde fixation step, nuclei were stained by Hoechst33342 (0.1 μg/ml) for 5 min and washed three times. The coverslips were mounted onto glass slides using mounting reagent and obtained image from fluorescence inverted microscope Axio Observer Z1 (Zeiss, Oberkochen, Germany) at 400x magnification.

12. Flow cytometry for cell cycle and apoptosis

The cell cycle was analyzed in cells transfected with siFAM188B by flow cytometry after staining with propidium iodide (Sigma-Aldrich). Annexin V assays for the detection of apoptotic populations was carried out using a BD FITC annexin V apoptosis detection kit I (BD Pharmingen, San Jose, CA, USA) according to the manufacturer's protocol.

13. Migration and invasion assay

Migration and invasion assays were performed according to the manufacturer's instructions. Cells (1×10^5) in 0.2 ml of serum-free medium were seeded onto the top of each Transwell in 24-well cell culture plates or/and 0.75 ml of complete growth medium containing 1% FBS was added to each well in the lower chamber. For invasion assay, Matrigel coating is needed before

addition of cells in Transwell. Following incubation for 48 hr at 37℃, non-invasive cells were removed from the upper chamber, the cells attached to the lower chamber were fixed and stained with Diff-Quik solution (Sysmex) and then counted under a light microscope.

14. Proteomic analysis and profiling of interacting partners

Immunoprecipitation of FAM188B interacting proteins were performed using HEK293 cells (1.5×10^6 cells/plate) transfected with 10 μg of pFLAG-FAM188B plasmid and incubated for 48 hr, and processed with 40 ul of anti-FLAG-M2 agarose affinity gel (Sigma-Aldrich) according to the manufacturer's protocol. Analyses were performed using an LTQ XL linear ion trap mass spectrometer (MS) system (Thermo Fisher Scientific). All MS/MS samples were analyzed using Proteome Discover software (version v.1.4; Thermo Fisher Scientific), set up to search the Uniprot_sprot database and IPI human database.

15. Immunocytochemistry

Cells were seeded on circular cover slip and fixed in 4% paraformaldehyde (PFA) for 15 min at room temperature (RT). After fixation step, wash two times with ice cold 1× PBS buffer and permeabilized in 1x PBS containing 0.25% Triton X-100 for 10 min at RT. The samples were blocked with 1% BSA in PBST buffer for 30 min and incubated primary antibody in humidified chamber

for 1 hr at RT. Dye-conjugated secondary antibodies were incubated for 1 hr at RT and nucleus was stained with Hoechst33342 (0.1 μg/ml) for 5 min. The coverslips were mounted onto glass slides using mounting reagent and images were captured using confocal microscope (Zeiss 510 Meta, Carl Zeiss).

16. Nuclear fractionation

Cells were transfected with siRNAs (NC and siFAM188B) and harvested at 24 hr and 48 hr. After washing the cell pellet with 1x PBS solution, harvested cells were lysed with 100 μl of Buffer A (10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.05% Igepal, pH7.9) for 10 min on ice. Supernatant was the cytoplasmic fraction and it was moved to new 1.5 ml tube. The pellet (3,000 rpm for 10 min at 4°C) was suspended with 75.2 μl of Buffer B (5 mM HEPES, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 26% glycerol (v/v), pH7.9) and 4.8 μl of 5 M NaCl. Homogenized the pellet with micro-pestle and leave it on ice for 30 min. Supernatant was aliquoted after additional centrifugation (24,000 g for 20 min at 4°C).

17. Luciferase assay

Dual luciferase assays were performed according to the manufacturer's protocol (Promega, Madison, WI, USA). Briefly, HCT-116 cells were transfected with NC siRNA or siFAM188B at a final concentration of 5 nM together with pGL2-p21-luc (Addgene, Cambridge, MA, USA) and

pGL4.70hRluc (Promega) plasmids. After 24 and 48 hr, cells were harvested, washed twice with cold 1x phosphate-buffered saline (PBS), and lysed using passive lysis buffer. Lysates were added to LARII solution layered on the bottom of a 96-well plate, and firefly luciferase activity was measured. A second measurement of Renilla luciferase activity was then performed by adding Stop&Go solution. All measurements were carried out using a Victor3 reader (Perkin Elmer, Waltham, MA, USA).

18. Chromatin immunoprecipitation assay

ChIP assays were performed using HCT-116 cells transfected with NC siRNA or siFAM188B at a final concentration of 5 nM and incubated for 48 hr. Cells were fixed in 1x PBS and 1% formaldehyde at 37°C for 10 min and stopped by adding glycine at a final concentration of 0.125 M. Fixed cells lysed with Igepal CA630 (Sigma-Aldrich) containing lysis buffer and centrifuged at 2,500 g for 5 min to collect nuclei. Collected nuclei were lysed and sonicated for 7.5 min (0.5 min on, 1 min off cycles) using a Bioruptor sonicator (Diagenode, Denville, NJ, USA). Fragmented genomic DNA was subsequently immunoprecipitated using an anti-p53 antibody (DO-1) with protein A/G agarose beads (Santa cruz Biotechnology). Immunoprecipitated DNA was purified by phenol/chloroform/isoamyl alcohol extraction. Amplification of *BAX* and *PUMA* genes was carried out using SYBR green master mix with p53-RE primers using an LC480 real-time PCR machine (Roche).

Amplification signals from immunoprecipitations were normalized to input control DNA.

19. p53 ubiquitination assay

HCT-116 cells were seeded 8.0×10^4 cells per well in 6-well plate and transfected with NC siRNA or siFAM188B. 100 μM of MG132 treated to siRNA-treated cells for 4 hr before harvest. Pellets were lysed with M-PER buffer (Thermo Scientific) with proteinase inhibitor (Roche) on ice for 1 hr. Cell lysates were immunoprecipitated with anti-p53 (DO-1) or anti-HA antibodies and immunoblotted with anti-HA or anti-p53 (DO-1) antibody, respectively. Samples were loaded 1 mg for IP lanes and 20 μg for whole cell lysate lanes.

20. Colony forming unit assay and soft agar assay

HCT-116 shRNA stable cells were plated at 1×10^3 cells/plate in a 6-well culture plate. Doxycycline treatment was used for induction of shFAM188B. On the seventh day after the shFAM188B induction, colonies were stained with crystal violet (Sigma) and images of visible colonies were obtained by fluorescence inverted microscope Axio Observer Z1.

HCT-116 cells (8.0×10^4 cells/well) were treated with NC siRNA or siFAM188B and incubated at 37°C 5% CO_2 containing incubator for 16 hr. HCT-116 cells were suspended in McCoy's 5A containing 0.35% low melting

agarose and plated onto solidified 0.6% agarose containing McCoy's 5A in six-well culture plates at a density of 5×10^3 cells per well. After incubating for 3 wk at 37°C in the 5% CO_2 incubator, the number of stained colonies was counted, and images were obtained. Three independent experiments were carried out for each assay.

21. Establishment of xenograft and tumor treatment *in vivo* with siFAM188B

Single-cell suspension of colon cancer cell line HCT-116 (2.0×10^6 cells) in 100ul of 1xPBS was subcutaneously injected in 6 wk old male BALB/c nude mice (CAnN.Cg-Foxn1nu/CrljOri, Orient Bio, Korea). When the tumor size reached $30mm^3$, Allstars negative control (Qiagen) or FAM188B (Qiagen) siRNA were injected into tumor *via* electroporation according to the manufacturer's instructions using NEPA21 Super Electroporator TypeII (Nepa Gene Co., Chiba, Japan). Electroporation was repeated every 7 days and monitored the tumor size twice a week for up to 3 wk. Each tumor volume was calculated by the modified ellipsoidal formula (Length x Width x Width) / 2. Length indicates the longest dimension of the tumor and width means shorter dimension, parallel to the mouse body. The experiment procedure and protocol were approved by Institutional Animal Care and Use Committee in National Cancer Center of Korea (NCC-16-231).

22. Immunohistochemical staining

Tumor tissues were fixed with 10% neutral buffered formalin. Formaldehyde-fixed specimens were paraffin-embedded and cut to a thickness of 3 μm. Sections were dried at 56°C for 1hr, and immunohistochemical staining was performed with Discovery XT (Ventana Medical Systems, Tucson, AZ, USA) as follows: sections were deparaffinized, rehydrated with EZ prep (Ventana Medical Systems), and washed with reaction buffer (Ventana Medical Systems). The antigens were retrieved with heat treatment in Tris-ethylenediaminetetraacetic acid (EDTA) pH 8.0 buffer (CC1, Ventana Medical Systems) at 90°C for 30 min for anti-FAM188B antibody (Atlas Antibodies AB, Stockholm, Sweden) and p53 (FL353). Parallel sections incubated with normal IgGs (SantaCruz) instead of primary antibodies were used as negative controls.

23. 3D structure prediction

FAM188B amino acid sequence was predicted and converted to PDB files at Phyre2 server (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index). The predicted FAM188B 3D model was visualized by PyMOL ver.1.7 program.

24. Statistical analysis

The statistical significance of differences between groups was determined using Student's *t*-test. ANOVA was used to analyze the clinicopathological

information in Prism software. Cox proportional hazard model was used to evaluate the effect of patients' characteristics on disease free survival and overall survival. The survival curves were estimated using Kaplan-Meire method, and the difference in survival curves was tested using the log-rank test. *P*-value less than 0.05 was considered statistically significant.

III. RESULTS

1. FAM188B is evolutionarily conserved in vertebrates

In the previous study, FAM188B was identified as a gene with significant differential exon usage between gastric cancer tissues and paired adjacent normal tissues in 30 gastric cancer patients based on comparisons of transcriptomes using an Affymetrix Exon 1.0ST microarray (NCBI Gene Expression Omnibus GSE30727).[36] However, FAM188B is annotated as 'a hypothetical protein' for which the transcript is the only supporting evidence for functionality in public databases. To characterize the function of FAM188B, FAM188B homolog sequences were searched in ncbi website (https://www.ncbi.nlm.nih.gov/protein) and aligned 16 FAM188B sequences from fish to human using EBI Clustal Omega.[35] Of the 16 species, all of them have highly conserved FAM188B catalytic residue in DUF4205 which is recently classified probable ubiquitin carboxyl-terminal hydrolase domain (Figure 1). Human is the most closely to primates such as *P. troglodytes* (chimpanzee), *P. abelii* (orangutan), *M. mulatta* (rhesus macaque) and *M. fascicularis* (crab-eating macaque) in the cladogram (Figure 2). Therefore, this result suggested that FAM188B would be vital gene in vertebrates and evolutionarily conserved.

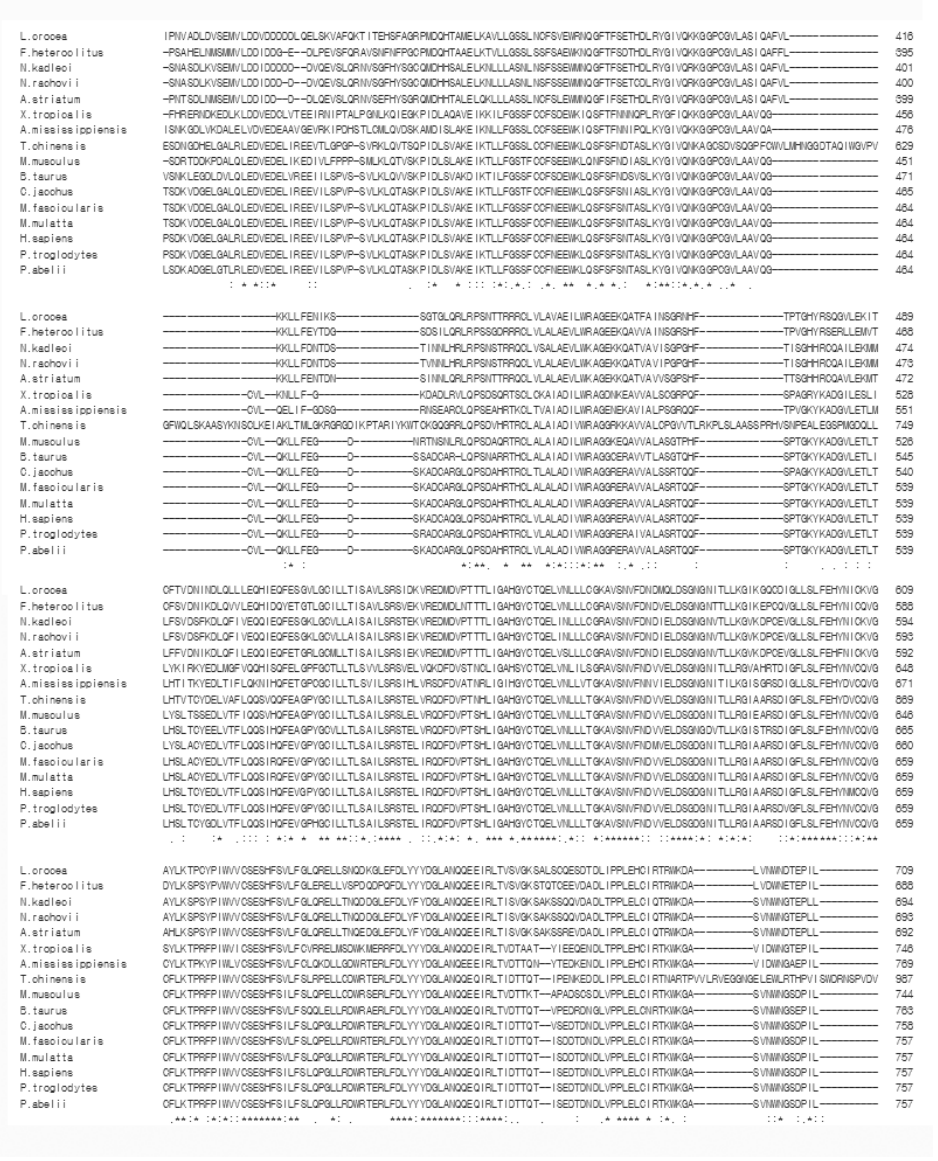


Figure 1. Alignments of FAM188B protein homolog sequences. Conserved residues are indicated with asterisks (completely conserved) or dots (mostly conserved).

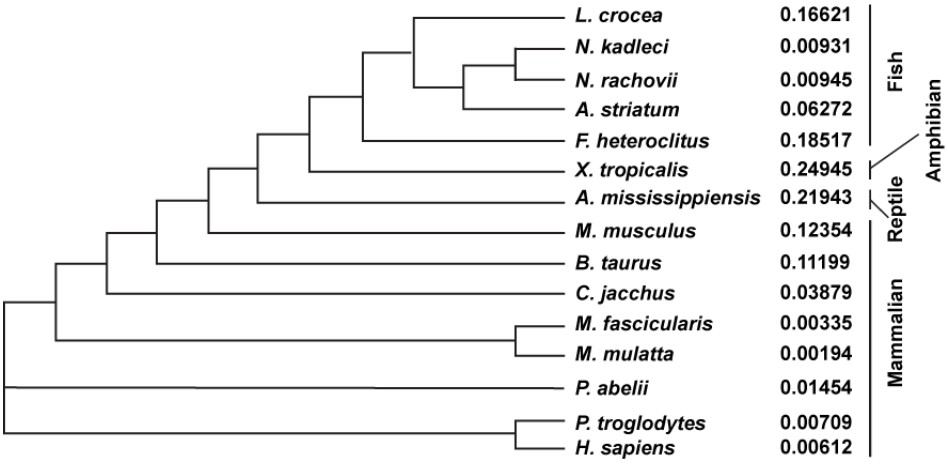


Figure 2. Cladogram based on the sequence alignment of 16 species.

2. FAM188B is confirmed genuine gene and elevated in tumor tissues

I previously investigated transcript variants in gastric cancer and found *FAM188B* among the numerous different exon usage genes.[36] To identify the existence of FAM188B, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed because this gene has been reported as hypothetical protein and not characterized in any species. *FAM188B* mRNA was detected in all 20 normal tissues and the mRNA expression was especially more elevated in lung, thyroid, trachea, and uterus (Figure 3A). Since FAM188B was picked from the differential gene expression analysis in gastric cancer, I carried out absolute qRT-PCR using 75 gastric cancer patient tissues (Table 2). Most of the patients showed that the fold change of *FAM188B* mRNA was greater than base line (68 out of 75 patients) (Figure 3B). Mean of *FAM188B* mRNA expression in tumor tissues also significantly increased compared to normal tissues (Figure 3C). Herein, FAM188B was confirmed genuine gene and it significantly increased in tumor tissues.

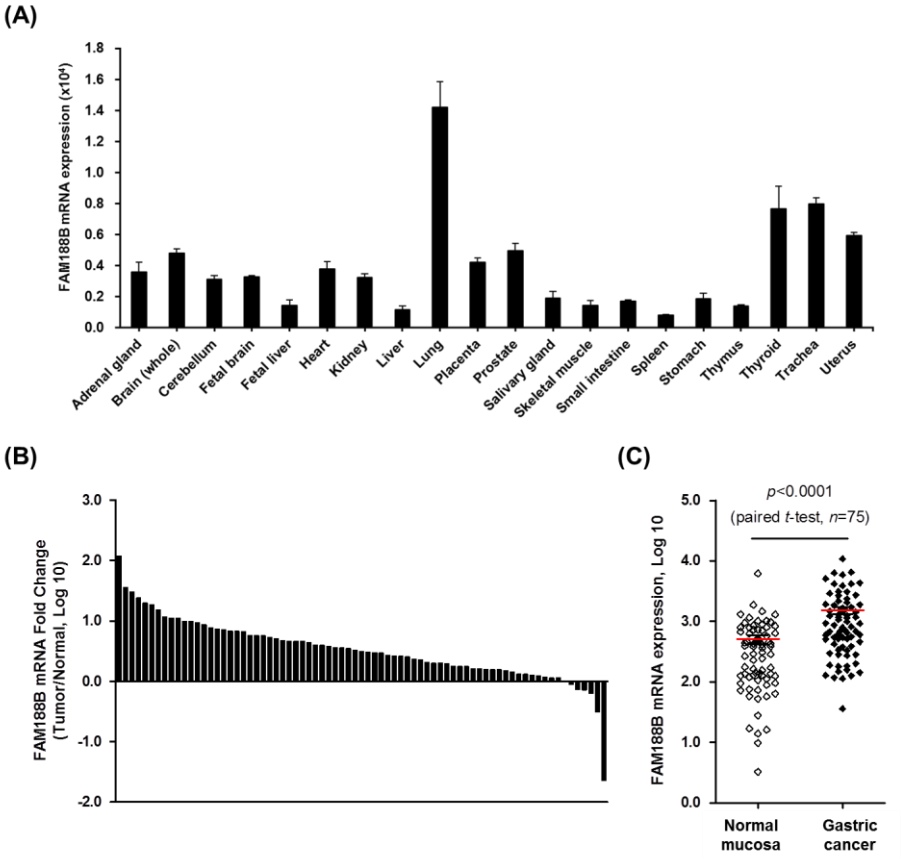


Figure 3. Validation of *FAM188B* mRNA expression in normal mucosa and gastric cancer tissues. (A) The mRNA expression was absolutely quantitated using qRT-PCR. Error bar indicates S.D. (B) Distribution of *FAM188B* mRNA fold change data was arranged in descending order. (C) The expression of *FAM188B* was absolutely measured by qRT-PCR. Red line indicates mean and error bar indicates S.D. All expriments were performed triplicate. (*n*=75)

Table 2. Clinicopathological information of 75 gastric cancer patients

			No. of patients
Number of patients	Total		75
	Male		53
	Female		22
Age at diagnosis (yr)	Range		21-86
	Mean ± SD		60.7±13.3
Disease stage†	Tumor stage	T1a	0
		T1b	1
		T2	16
		T3	29
		T4a	22
		T4b	7
	Node stage	N0	26
		N1	11
		N2	13
		N3a	17
		N3b	8
	Metastasis stage	M0	63
		M1	12
Lauren type	Intestinal		33
	Diffuse		25
	Mixed		7
	Intermediate		8
	Not annotated		2
Bormann type	Type I (protruded type)		1
	Type II (ulcerative type)		21
	Type III (ulceroinfiltrated type)		46
	Type IV (diffuse type)		6
	Not determined		1

†Stage classification follows the TNM classification system by Union for International Cancer Control (UICC)

3. High expression of *FAM188B* mRNA in gastric cancer shows significant differences in Lauren classification

To further analyze the mRNA expression of *FAM188B*, I used clinicopathological classification to compare them, such as Lauren classification, Histology, Bormann type, and stages (pT stage, pN stage, final stage) (Table 3). Among the classification methods, *FAM188B* mRNA expression showed significant differences in Lauren classification. Two main types of Lauren classification (intestinal and diffuse type) were analyzed and mRNA expression of *FAM188B* was significantly increased in Intestinal type. To examine whether FAM188B was used as a marker to identify stages, I plotted the FAM188B expression level of two classified stages (early gastric cancer and advanced gastric cancer) (Figure 4). *FAM188B* mRNA level was higher in the tumor tissues of advanced gastric cancer (stage IIa ~ IV) compared to that of early gastric cancer. FAM188B expression was increased in the tumors but statistically significant only in IIb, IIIa and IV stages. In summary, FAM188B may be used as a possible marker of advanced gastric cancers (AGC) and it would be involved in the progression from early gastric cancer to advanced gastric cancer.

Table 3. Analysis of *FAM188B* mRNA expression by clinicopathological information

	Class	Mean	*p*-value	Significance
Lauren classification	Intestinal	7.18542	0.0079	**
	Diffuse	2.43012		
	Mixed	2.29971		
	Indeterminate	20.17803		
Histology	Well diff.	6.48604	0.7132	N.S.
	Moderate diff.	4.71920		
	Poorly diff.	10.09003		
	Signet ring	3.04266		
	Mucinous	2.72247		
	Not annotated	10.97892		
Bormann type	Bormann 1	3.73687	0.8451	N.S.
	Bormann 2	10.24630		
	Bormann 3	6.11695		
	Bormann 4	5.53745		
	Not identified	1.76495		
pT stage	T1b	1.76495	0.2576	N.S.
	T2	3.89326		
	T3	11.62397		
	T4a	3.93559		
	T4b	2.90383		
pN stage	N0	8.26654	0.3374	N.S.
	N1	4.57596		
	N2	13.09295		
	N3a	3.99201		
	N3b	1.55150		

final stage	Ia	1.76495	0.643	N.S.
	Ib	3.61544		
	IIa	10.21396		
	IIb	7.56976		
	IIIa	13.12077		
	IIIb	4.44903		
	IIIc	2.64522		
	IV	3.68534		

† Well diff: well differentiation, Moderate diff: moderate differentiation, Poorly diff: poorly differentiation, p: pathologic stage, T: characteristics of the tumor, N: presence of any lymph nodes metastases, N.S.: Not significant

§ Statistical significance was calculated by ANOVA in GraphPad Prism5 program. (*p<0.05, **p<0.01, ***p<0.001)

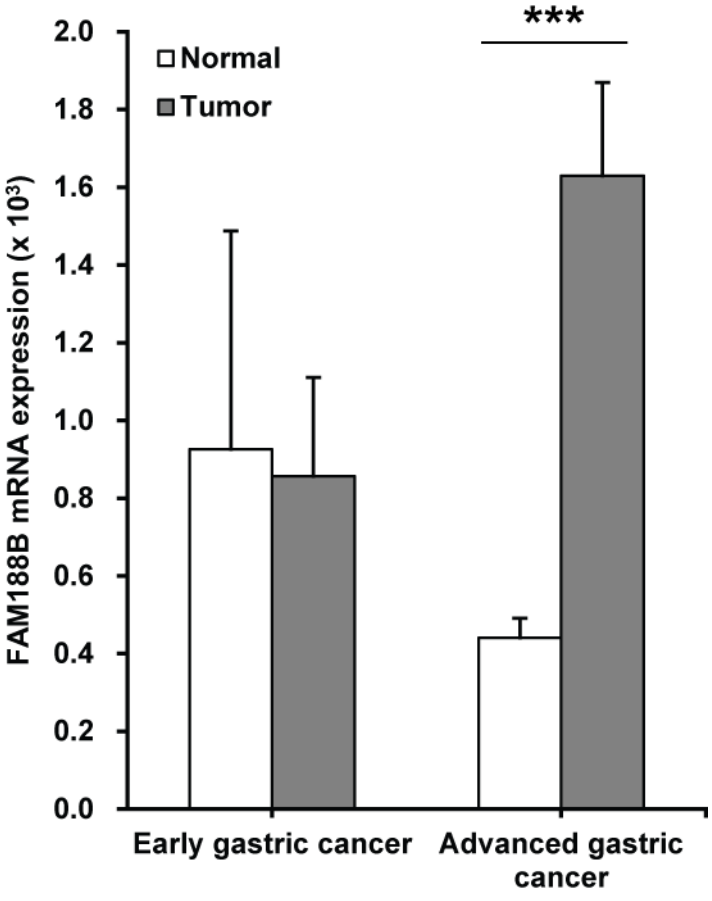


Figure 4. *FAM188B* mRNA expression analysis in early gastric cancer and advanced gastric cancer. Absolutely quantitative RT-PCR results were analyzed by stage classification. Mean±S.E.M. Stage classification follows the TNM classification system by Union for International Cancer Control (UICC), (Early gastric cancer (EGC): stage Ia and Ib (n=10), Advanced gastric cancer (AGC): Stage IIa, IIb, IIIa, IIIb, IIIc, IV (n=65)). *p<0.05, **p<0.01, ***p<0.001

4. Generation of FAM188B antibody

After verification of the mRNA expression of *FAM188B* in tissues, I tried to quantify the FAM188B protein expression. However, among the commercially available antibodies, there were no antibodies that could detect the protein by Western blot. Therefore, the epitope of FAM188B was predicted and injected it into the rabbit to generate our own anti-FAM188B antibody (Figure 5A). Of the several candidates, only the FAM188B (F374C) antibody targeting C-terminus of the protein detected a properly sized FAM188B protein. To confirm that the antibody works properly, GFP-tagged FAM188B constructs were transfected into HEK293 cells and detected by FAM188B (F374C) antibody (Figure 5B). Only the FAM188B N-terminus tagged GFP constructs (pAcGFP-C1-FAM188B) was detected by GFP (JL-8) antibody. According to this result, I compared the membrane detected with GFP antibody and the membrane detected with FAM188B (F374C) antibody. The size shifted protein in a lane of pAcGFP-C1-FAM188B except endogenous FAM188B was confirmed and they were in the same position at the membranes (Figure 5B). Taken together, FAM188B (F374C) antibody was appropriately established since same size proteins were found in these two membranes.

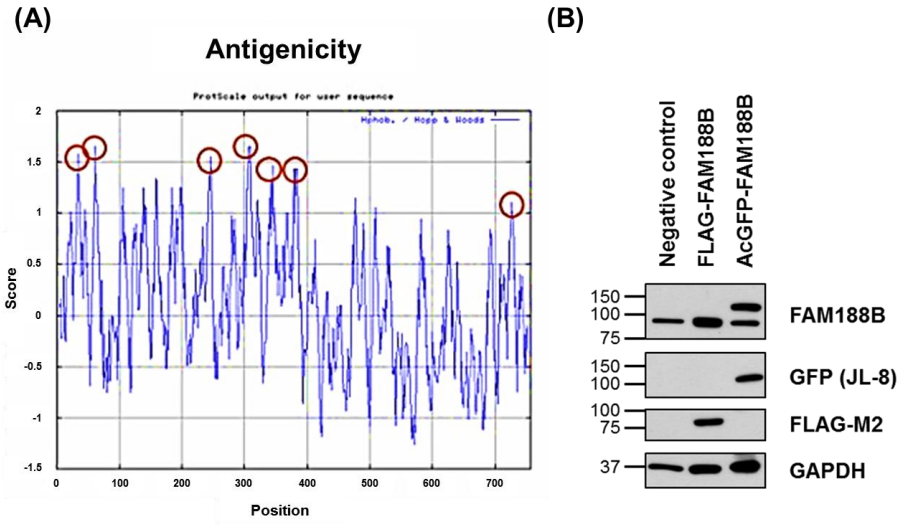


Figure 5. FAM188B epitope prediction and antibody validation by Western blot. (A) Antigenicity prediction of FAM188B. Red circles indicate selected peptides for epitope. (B) HEK293 transfected with GFP-tagged FAM188B and AcGFP-tagged FAM188B were separated by SDS-PAGE and detected by FAM188B, FLAG-M2 and GFP antibodies.

5. Knockdown of FAM188B results in apoptotic cell death

To identify the function of FAM188B, *FAM188B*-targeting siRNA was treated in AGS to knockdown the expression. I used two siRNAs targeting FAM188B which target the exon 16 to 17 (siFAM188B) and exon 5 (siFAM188B-2) due to avoid off-target effect of siRNA (Figure 6A). I carried out qRT-PCR to confirm the mRNA expression in AGS treated with siFAM188B from 24 hr to 96 hr after siRNA treatment. Treatment of siRNA resulted in a significant decrease in the mRNA expression of FAM188B at all time points. The mRNAs in siFAM188B treatment only expressed 30% of NC siRNA (Figure 6B). Next, I performed Western blot analysis to investigate whether protein expression was decreased as decrement of mRNA expression. Compared to NC siRNA, siFAM188B treatment dramatically reduced the expression of FAM188B from the 24 hr time point (Figure 6C). Therefore, siFAM188B appropriately knocked down *FAM188B* mRNA and subsequently reduced protein expression.

As a result of siFAM188B treatment, slow proliferation and suspended dead cells in AGS were observed (Figure 7A). I hypothesized that FAM188B relates to cell survival. To test this hypothesis, cell cycle analysis and nuclei staining assay with siFAM188B were performed. I observed increment of G0/G1 phase and nuclei fragmentation in siFAM188B treated condition, suggesting that FAM188B regulates the cell survival (Figures 7B and 7C). In order to elucidate the mechanism of cell death, apoptosis was measured by Annexin V assay.

Since Annexin V binds to the endothelium phosphatidylserine (PS) and propidium iodide (PI) was able to penetrate the nucleus through perforated membranes during apoptosis, cells were stained with Annexin V and PI. Annexin V/PI stained cells were increased in FAM188B knocked down cells (31.47%) compared to negative control (11.53%) (Figure 7D). Taken together, siFAM188B affects reduction of mRNA and protein expression level and results in apoptotic cell death, suggesting that FAM188B regulates cell survival.

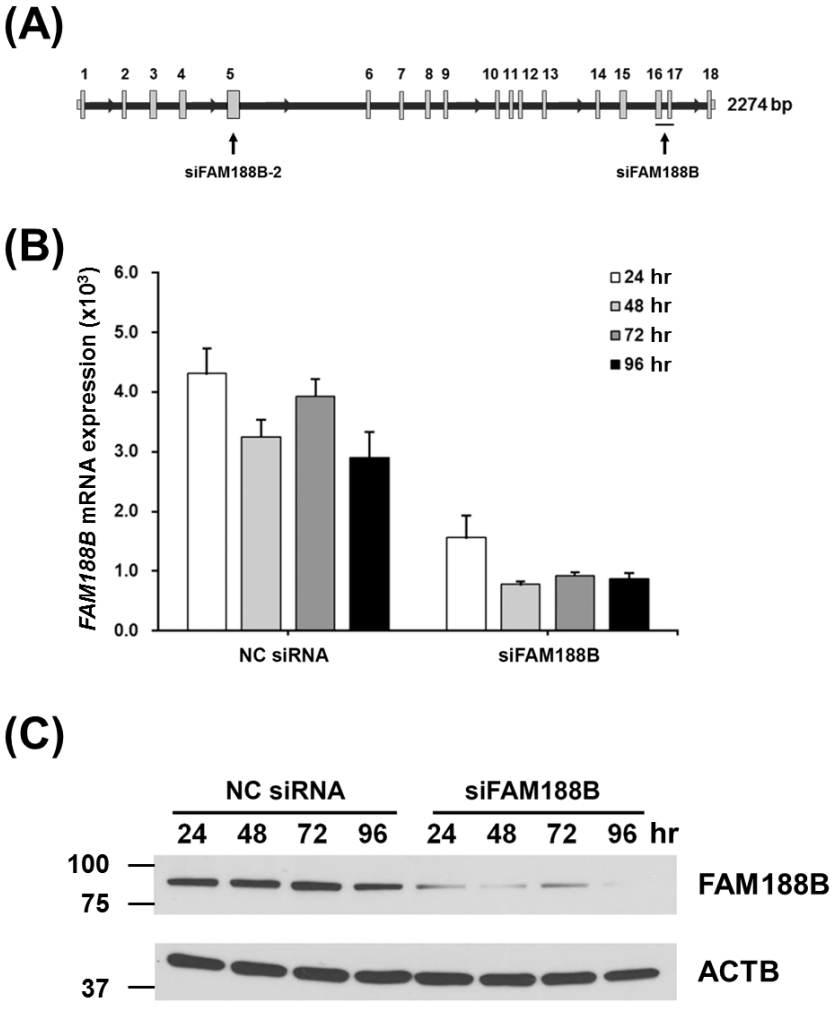


Figure 6. FAM188B expression validation in gastric cancer cell line AGS treated with siFAM188B. (A) Schematic view of FAM188B and siFAM188B targeting regions. (B) mRNA expression was absolutely quantitated by qRT-PCR. All experiments were performed triplicate. Error bar indicates S.D. (C) Cell lysates were separated by SDS-PAGE and detected by anti-FAM188B and anti-ACTB antibodies.

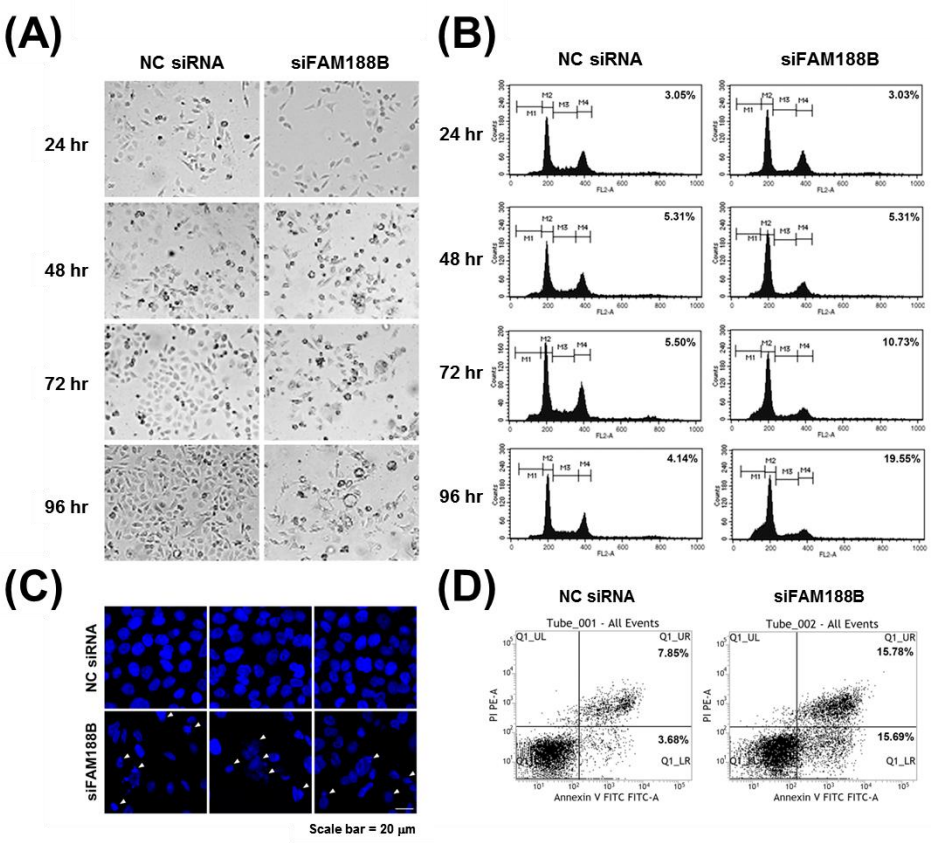


Figure 7. Knockdown of *FAM188B* results in cell death. (A) Phenotype of AGS treated with or without siFAM188B. (B) Cells were processed for cell cycle analysis by flow cytometry (dead cell population: sub G0/G1 is indicated in %). (C) DNA stained by Hoechst33342 with or without siFAM188B. White arrow heads indicate fragmented nuclei. Scale bar = 20 μm. (D) Cells were transfected with siFAM188B and stained with Annexin V/PI. The stained cells were analyzed by flow cytometry for apoptosis. UR and LR indicates apoptotic cells.

6. Knockdown of FAM188B reduces migratory potential and invasive property

Most of the causes of death in patients with solid tumors are metastatic relapses. The initial phenotype of metastasis is invasiveness of the cell.[37] Therefore, it is important to know the acquirement of invasiveness in cancer cell. To examine whether FAM188B has migratory potential and/or invasive property, I performed migration and invasion assays *in vitro*. Migrated cells through the pore were counted and the results were plotted. The migratory potential was significantly decreased in AGS treated with siFAM188B-1 and -2 but there was no difference between siFAM188B treatments (Figure 8A). Knockdown of FAM188B also significantly inhibited invasive property in AGS cells but there was a significant difference between siFAM188B treatments (Figure 8B). Taken together, FAM188B regulates migration and is involved to metastatic relapse.

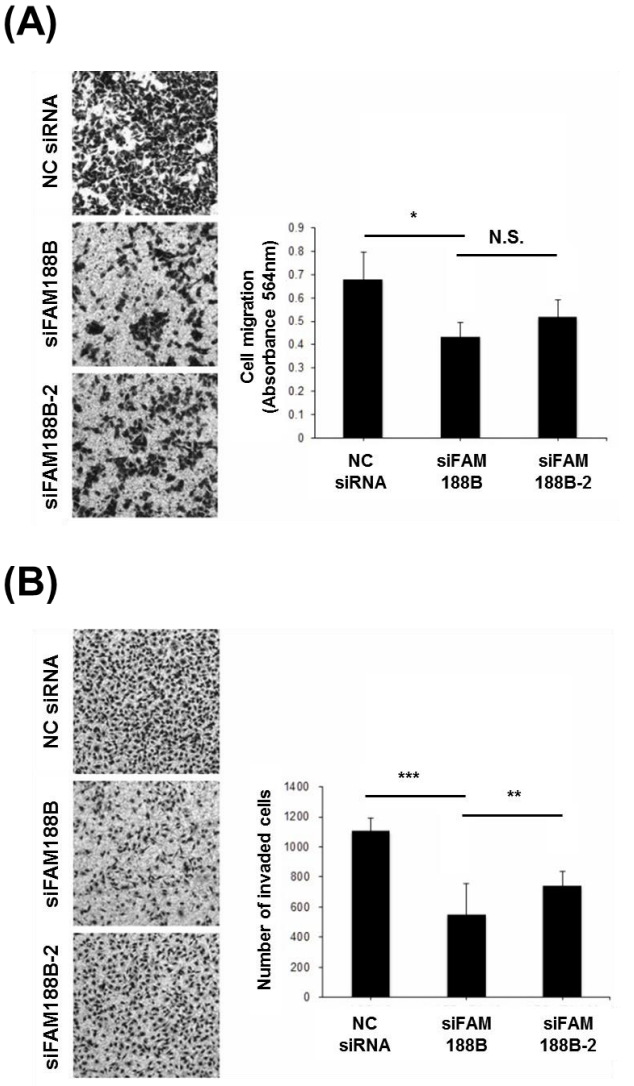


Figure 8. Inhibitory effect of siFAM188B on migration and invasion ability.

(A) Cells were treated with or without siFAM188B and counted the number of migrated cells. (B) Cells were treated with or without siFAM188B and counted the number of invaded cells. N.S.: not significant, *p<0.05, **p<0.01, ***p<0.001

7. FAM188B is localized in the cytoplasm

Previous results indicate that FAM188B is increased in tumor tissues and cell death in knockdown. To apply the findings to therapy, I have to know where FAM188B is expressed in the cell. Therefore, GFP-tagged FAM188B was transfected into HEK293 cells and images were obtained from fluorescence inverted microscope (Figure 9). According to the images, FAM188B was localized in cytoplasm. To confirm whether cytoplasmic localization of FAM188B is specific to HEK293 or exogenous protein, I performed immunocytochemistry on endogenous FAM188B by anti-FAM188B (F374C) antibody. Three gastric cancer cells (AGS, SNU-638, and MKN-28), a colon cancer cell HCT-116, and a non-cancerous cell HEK293 were used. FAM188B was located in the cytoplasm in the all tested cells (Figure 10). Therefore, I identified FAM188B localized in cytoplasm in several cells and I would have to use the chemicals to treat the tumor because of the limitation of access.

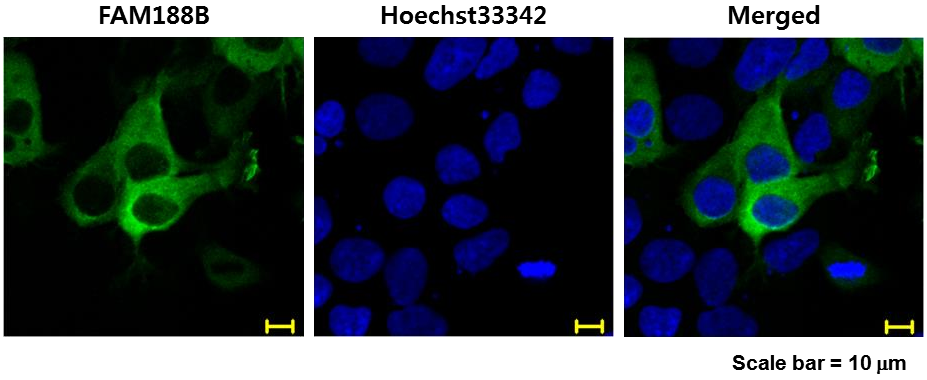


Figure 9. Location of exogenous GFP tagged FAM188B in HEK293. GFP-tagged FAM188B was transfected in HEK293 cells and image was obtained from fluorescence inverted microscope. Hoechst33342 was used as DNA staining reagent. Scale bar = 10 μm

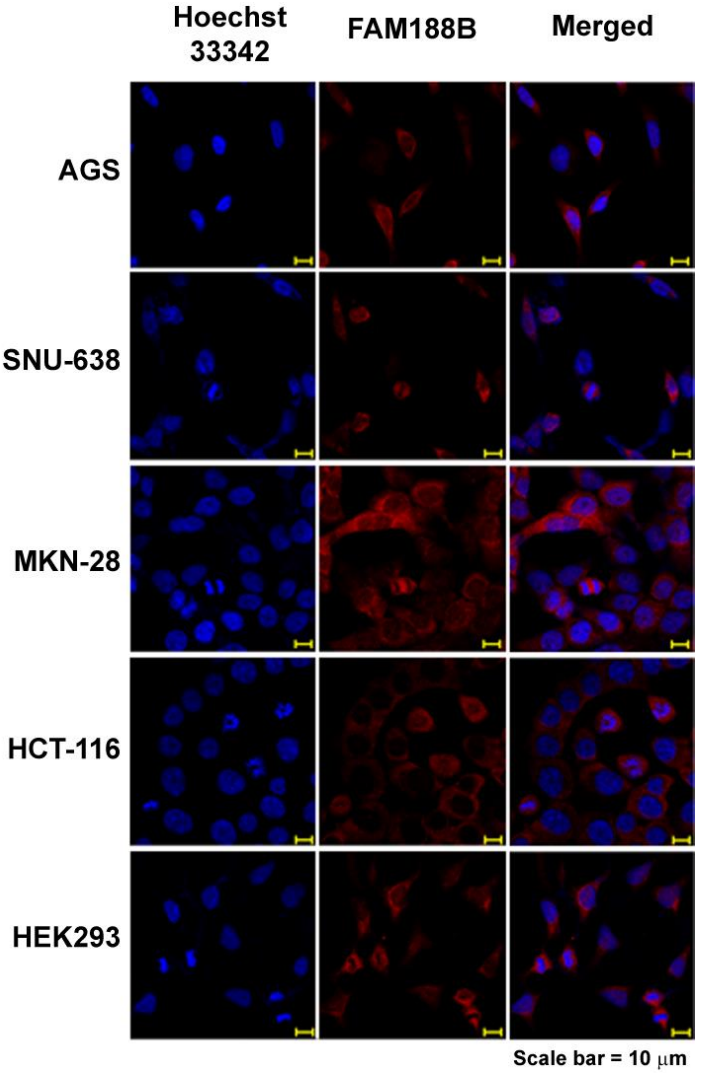


Figure 10. Localization of endogenous FAM188B in cell lines. FAM188B was stained with Alexa Fluor 594 dye conjugated anti-FAM188B antibody and images were obtained by fluorescence inverted microscope. Hoechst33342 indicates nucleus. Scale bar = 10 μm

8. Treatment of chemotherapeutic agents is more effective in FAM188B down-regulated cells

Cisplatin and etoposide are the most widely used anti-cancer drugs for chemotherapy. Cisplatin crosslinks with the purine base on DNA to interfere with the DNA repair mechanism and causes apoptosis.[38] Etoposide also causes DNA strand breakage due to form the ternary complex with DNA and Topoisomerase II enzyme.[39] To examine the effect of anti-cancer drugs, I analyzed cell survival after cisplatin or etoposide treatment in AGS treated with or without siFAM188B by using MTT assay. Two anti-cancer drugs significantly reduced the cell survival rate in siFAM188B treated cells (Figure 11A and 11B). Therefore, chemotherapeutic agents were more effective in FAM188B down-regulated condition. This suggests that it can be applicated in gastric cancer, which shows low FAM188B expression, as a possible mediation.

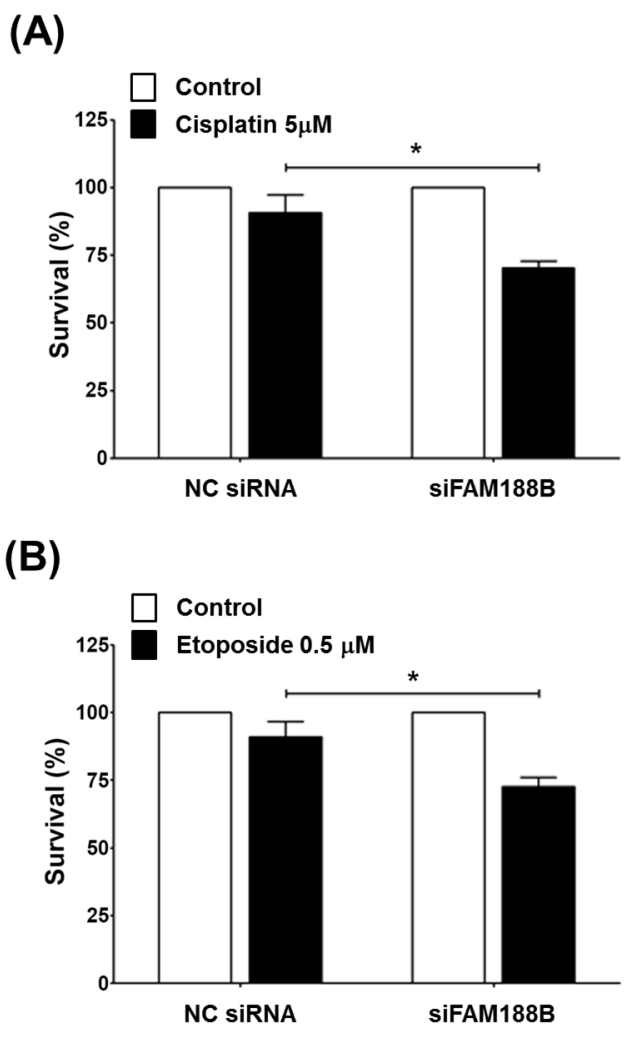


Figure 11. Effects of anti-cancer drugs on cell survival of AGS cells treated with siFAM188B. (A) Cells were simultaneously treated with cisplatin and siFAM188B. (B) AGS cells were simultaneously treated with etoposide and siFAM188B. Cell survival was measured by MTT assay. All experiments were performed triplicate. Error bar indicates S.D. * $p<0.05$

9. FAM188B is related to resistance to chemotherapeutics

Using immunohistochemistry of tissue microarrays, the expression of FAM188B protein was analyzed in gastric cancer lesions in a total of 107 gastric cancer patients (Table 4). Fundic glands showed strong positivity, and pyloric glands, foveolar epithelium and intestinal metaplasia showed moderate to weak signals. The staining pattern was mostly cytoplasmic with occasional luminal surface staining. Representative immunostained images showing 'High' and 'Low' FAM188B expression in gastric cancer are presented in Figure 12A.

A statistical analysis revealed that FAM188B expression was significantly associated with the sex of the patient and the size and pathological classification of the lesion (Table 3). High expression was more frequent in males (86.1%, n=31) than in females (13.9%, n=5) (p=0.014), and lesions were larger in the FAM188B low-expression group (5.95±2.84 cm) than in the high-expression group (4.88±1.87 cm) (p=0.045). Notably, differences in pathological characteristics between FAM188B 'Low' and 'High' groups were also significant, based on differentiation status by Lauren classification (FAM188B high: 61.1% in intestinal type, 25.0% in diffuse type; p<0.001). However, FAM188B expression itself as a prognostic factor showed no significance in terms of either disease-free survival (p=0.585) or overall survival (p=0.388) (Figure 12B). Thus, I assessed whether the efficacy of chemotherapy depended on FAM188B expression. Intriguingly, both disease-free and overall survival were improved by adjuvant chemotherapy in the FAM188B 'Low' group

(Figure 12C), but no survival benefit of chemotherapy was observed in the FAM188B ‘High’ group (Figure 12D). The consistent result was found after adjusting the effects of other prognostic factors. These findings suggest that FAM188B expression can be used as a predictive marker of chemotherapeutic efficacy.

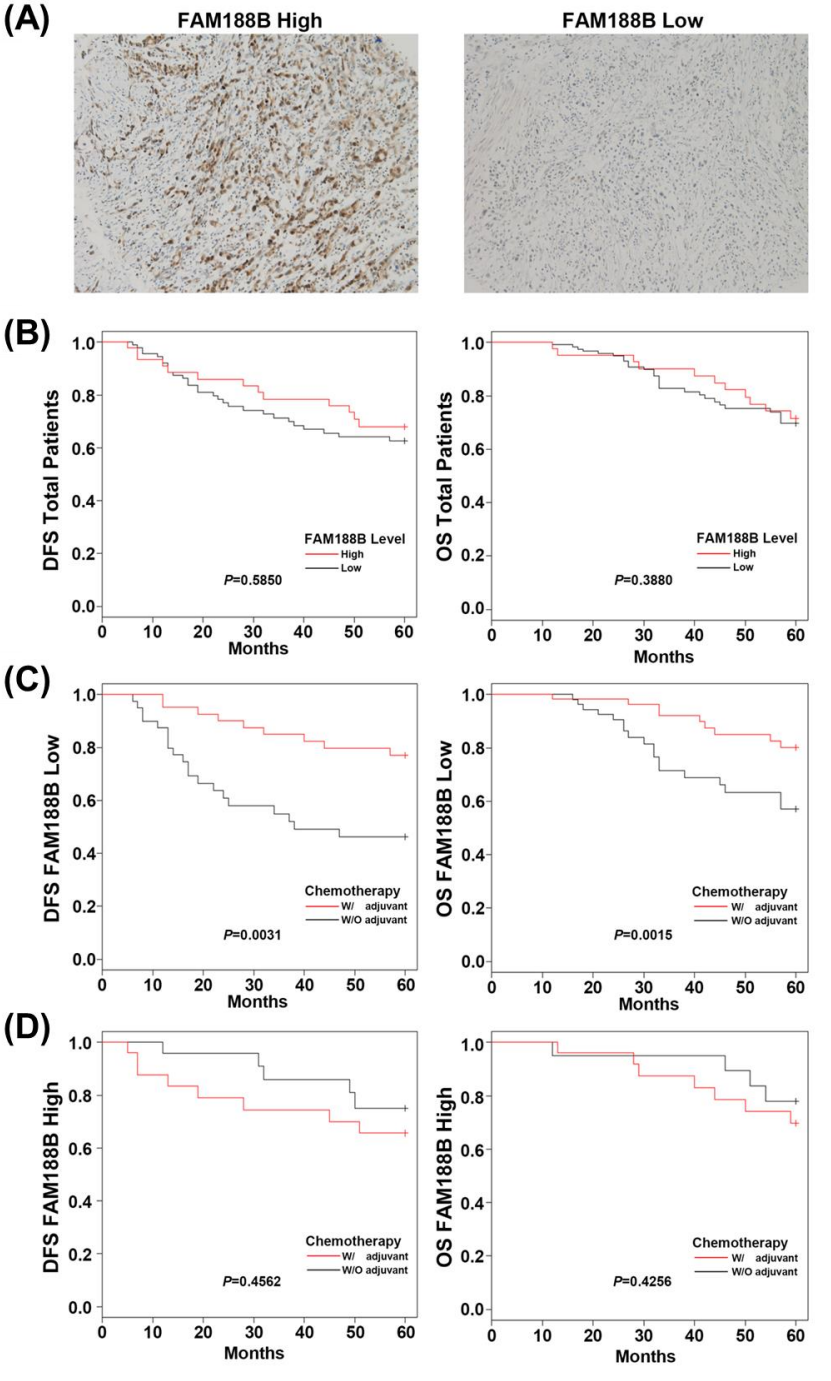


Figure 12. FAM188B resists to chemotherapeutic agents. (A) Immunohistochemistry of gastric cancer tissues. Representative images of FAM188B 'High' (left panel) and FAM188B 'Low' (right panel) are presented. (B) Differences in disease-free survival and overall survival were not statistically significant between FAM188B 'Low' patients and FAM188B 'High' patients (p=0.585 and p=0.388 for disease-free and overall survival, respectively). (C) The dependence of differences in disease-free survival and overall survival of FAM188B 'Low' patients on adjuvant chemotherapy. Patients receiving adjuvant therapy showed higher disease-free survival (p=0.0031) and overall survival (p=0.0015) than those than without adjuvant chemotherapy. (D) The dependence of differences in disease-free survival and overall survival of FAM188B 'High' patients on adjuvant chemotherapy. There was no significant difference in disease-free survival (p=0.4562) or overall survival (p=0.4256) between patients receiving and not receiving adjuvant therapy.

Table 4. Relationship between FAM188B expression and clinicopathological characteristics in 107 gastric cancer patients

	FAM188B 'Low' expression (*n*=71)	FAM188B 'High' expression (*n*=36)	*P*-value
Sex			0.014
Male	45 (63.4%)	31 (86.1%)	
Female	26 (36.6%)	5 (13.9%)	
Age (yr)	53.8±10.7	56.2±11.6	0.294
Size (cm)	5.95±2.84	4.88±1.87	0.045
Location			0.179
Upper body	17 (23.9%)	5 (13.9%)	
Middle body	31 (43.7%)	13 (36.1%)	
Lower body	23 (32.4%)	18 (50.0%)	
Pathological Classification			
Lauren type			< 0.001
Intestinal	18 (25.4%)	22 (61.1%)	
Diffuse	45 (63.4%)	9 (25.0%)	
Mixed	8 (11.3%)	5 (13.9%)	
T-stage			0.099
1-2	13 (18.3%)	13 (36.1%)	
3	30 (42.3%)	14 (38.9%)	
4	28 (39.4%)	9 (25.0%)	
N-stage			0.352
0-1	27 (38.0%)	15 (41.7%)	
2	19 (26.8%)	13 (36.1%)	
3	25 (35.2%)	8 (22.2%)	

	FAM188B 'Low' expression (*n*=71)	FAM188B 'High' expression (*n*=36)	*P*-value
Stage			0.603
II	24 (33.8%)	14 (38.9%)	
III	47 (66.2%)	22 (61.1%)	
Adjuvant Chemotherapy			0.247
Not done	36 (50.7%)	14 (38.9%)	
Done	35 (49.3%)	22 (61.1%)	
Recurrence in 5 yr			0.630
Absent	46 (64.8%)	25 (69.4%)	
Present	25 (35.2%)	11 (30.6%)	
Death in 5 yr			0.445
Deceased	44 (62.0%)	25 (69.4%)	
Survived	27 (38.0%)	11 (30.6%)	
Recur. or Death in 5 yr			0.636
Recurred	42 (59.2%)	23 (63.9%)	
Non-recurred	29 (40.8%)	13 (36.1%)	

10. FAM188B is expressed in other cancer cell lines and tissues.

To determine the expression of FAM188B in other cancers, I first searched for mRNA expression in the Cancer Cell Line Encyclopedia (CCLE) database.[23] Most of solid cancer cell lines express high *FAM188B* mRNA except lymphoma, leukemia, B-cell, T-cell, and chondrosarcoma (Figure 13A). Next, mRNA expression level was confirmed in various cancer cell lines and found that colon cancer cell lines (HCT-116, SW620 and HT-29) and breast cancer cell lines (JIMT1 and MDA-MB-231) highly expressed *FAM188B* mRNA (Figure 13B). The protein expression pattern of endogenous FAM188B in several cell lines was similar to mRNA expression (Figure 13C). These results proposed that the existence of hypothetical protein FAM188B was confirmed experimentally and it is highly expressed in colorectal and breast cancer cells.

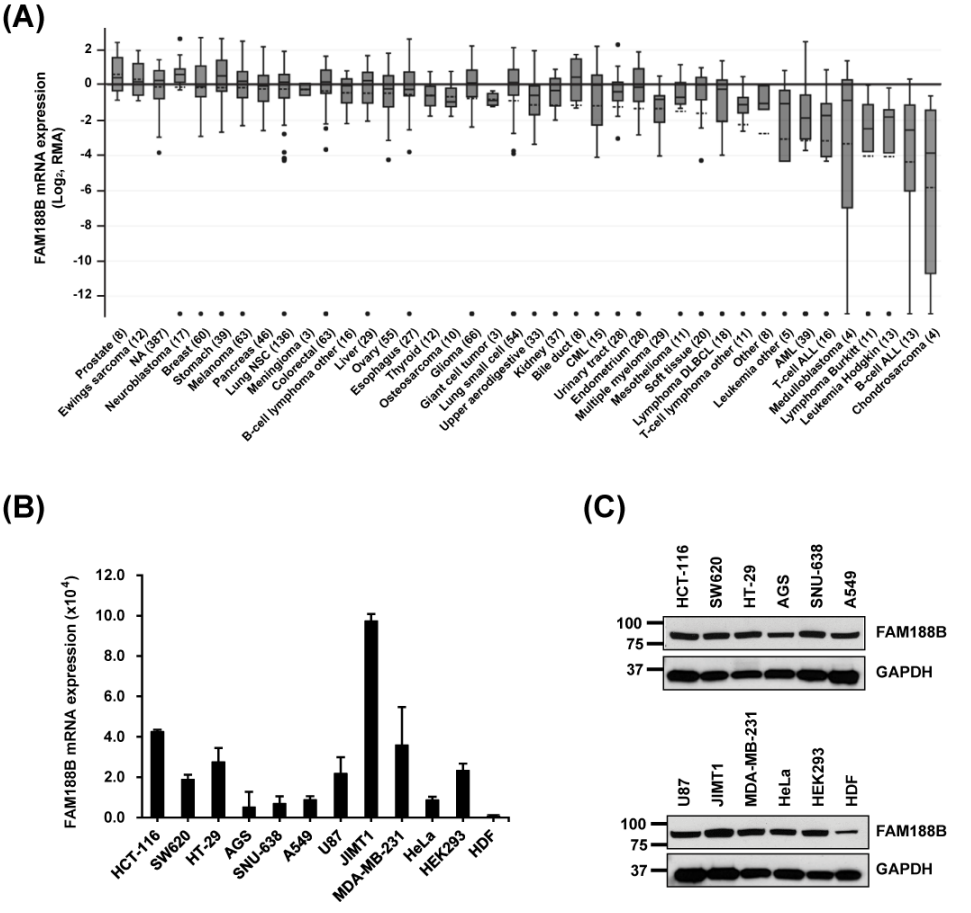


Figure 13. Confirmation of *FAM188B* mRNA and protein expression level.

(A) *FAM188B* mRNA expression levels in CCLE database. Box-and-whisker plots show the distribution of mRNA expression for each cancer type. Line in the box indicates median and dashed line indicates mean. (B) mRNA of FAM188B was absolutely quantitated by qRT-PCR using 12 cell lines. Error bar indicates S.D. (C) Whole cell lysates were separated with SDS-PAGE and detected by anti-FAM188B and anti-GAPDH antibodies.

11. FAM188B is highly expressed in colon cancer tissues.

According to the previous results, I found that FAM188B is highly expressed in gastric cancer and knockdown of FAM188B results in apoptosis and additional chemotherapy significantly reduced cell survival. Since FAM188B also expressed in other cell lines, especially in colon and breast cancer cell lines, public database Oncomine (www.oncomine.org)[26] was used to search the mRNA expression of tumor tissues. Of the twenty other cancer types, *FAM188B* mRNA expression increased only in 3 colon cancer analysis sets (Figure 14A). To examine in more detail, I investigated the expression of FAM188B in each of the three datasets. *FAM188B* mRNA expression was highly increased in multiple CRC data sets[24, 25] (TCGA Colorectal 2, p=2.48×10-16; GSE20916, p=6.59×10-7; GSE20842, p=2.68×10-26) (Figure 14B). These data confirmed that FAM188B is up-regulated in colorectal cancer tissues. Therefore, I decided to identify the function of FAM188B in colorectal cancer.

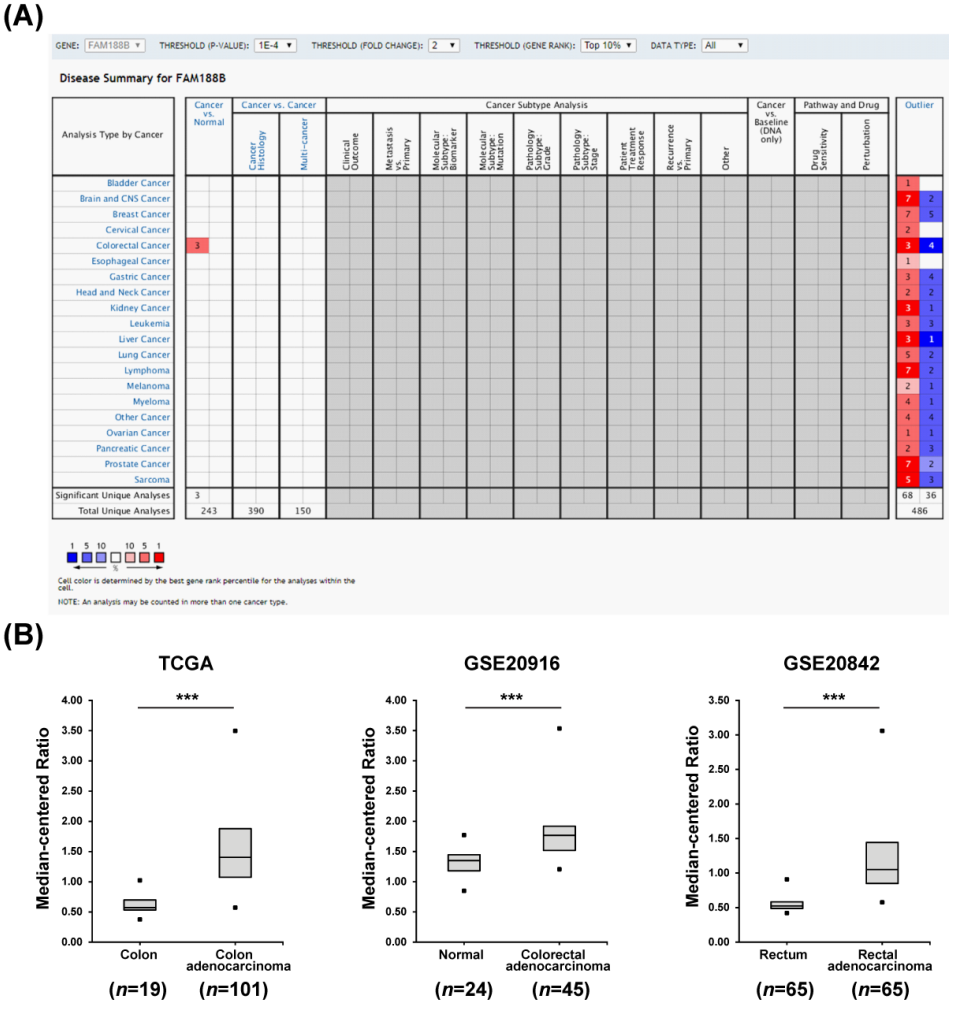


Figure 14. Analysis of FAM188B expression in 20 distinct cancer types. (A) RNA-seq. analyses of FAM188B were displayed in Oncomine webpage. (B) FAM188B expression comparison between normal and tumor patient tissues using TCGA colorectal 2, GSE20916, and GSE20842 data set. Upper and lower dots indicate the maximum and minimum values. The middle line in the box indicates median.

12. FAM188B down-regulation leads to cell death in colon cancer cell lines.

To understand the function of FAM188B, I knocked down FAM188B expression using *FAM188B*-specific siRNA in the three colon cancer cell lines HCT-116, HT-29 and SW620 because these cell lines have different mutations. From the COSMIC Cell Line Project, HT-29 and SW620 cell lines have p.R273H mutation but not in HCT-116. K-ras mutation was detected in HCT-116 and SW620 cell line.[40] Transfection of cells with siFAM188B decreased FAM188B mRNA expression from 24 hr to 96 hr as determined by qRT-PCR (Figure 15A, Figure 16A and 16E) whereas negative control (NC) siRNA had little effect on FAM188B expression. FAM188B protein levels were also reduced by siFAM188B, as determined by Western blot (Figure 15B, Figure 16B and 16F). To test the effects of FAM188B knockdown on cell growth, two different FAM188B-targeting siRNAs (siFAM188B and siFAM188B-2) were used. After knockdown of FAM188B by siFAM188B or siFAM188B-2, HCT-116 cell growth decreased with time (Figure 15C, Figure 16C and 16G). A microscopic analysis of DAPI-stained cells also revealed that siFAM188B treatment increased brightly fluorescent and fragmented nuclei, which is indicative of apoptosis (Figure 15D). As cell growth inhibition could be occurred either by cell cycle arrest or cell death, I examined the effect of FAM188B knockdown on cell cycle. Interestingly, the sub-G0/G1 population increased in the siFAM188B-treated cells, which corresponds to apoptotic cells (Figure 15E). In addition, FAM188B knockdown increased the population of

Annexin V/PI–stained cells. Apoptosis induced by siFAM188B was not limited to HCT-116 cells because siFAM188B treatment also increased Annexin V/PI-positive cells in other colon cancer cell lines, HT-29 and SW620 compared with NC siRNA-treated cells (Figure 15F, Figure 16D and 16H). All these data suggest that down-regulation of FAM188B expression in colon cancer cells leads to apoptosis.

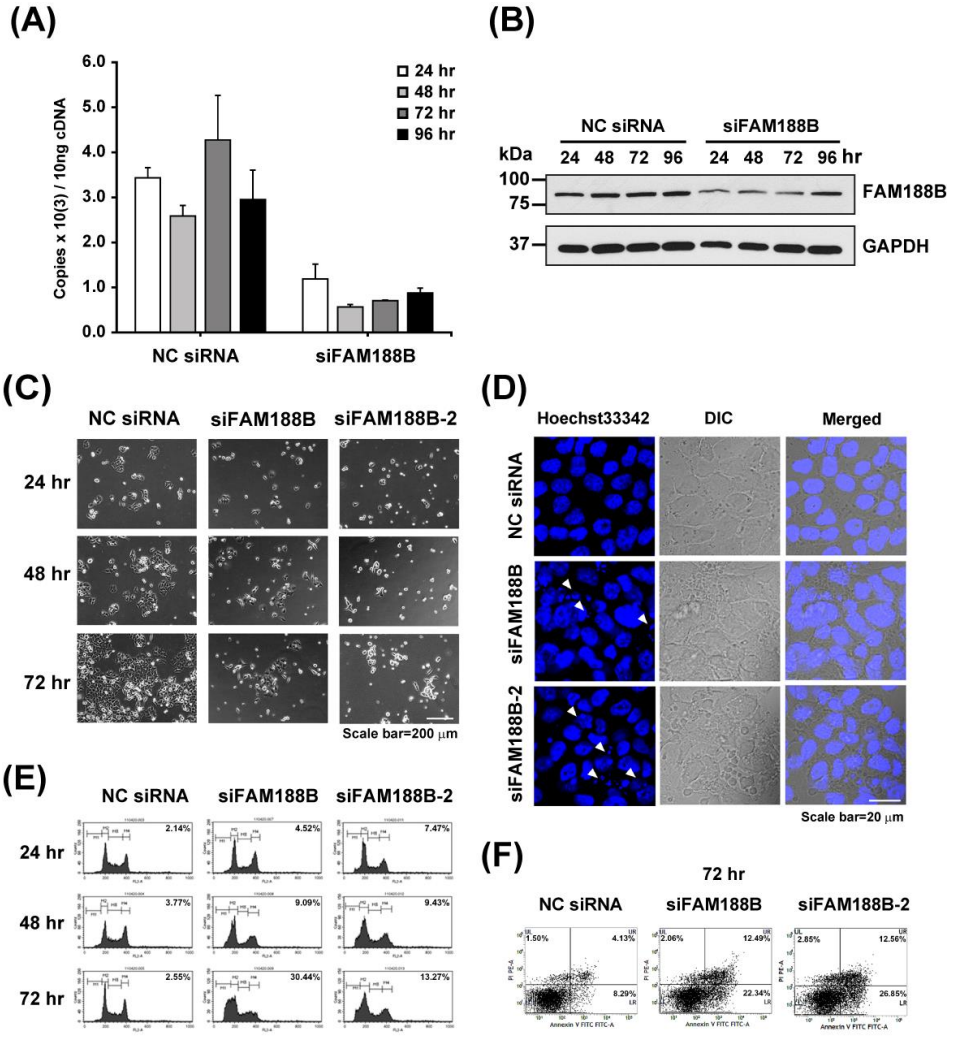


Figure 15. Effects of FAM188B knockdown on cell death in HCT-116. (A) mRNA level of *FAM188B* on HCT-116 cells treated with negative control siRNA or siFAM188B was absolutely quantitated by qRT-PCR. Error bar indicates S.D. (B) Lysates were separated by SDS-PAGE and detected by anti-FAM188B antibody and anti-GAPDH antibody as an internal control. (C) HCT-116 cells were transfected with NC siRNA, siFAM188B, or siFAM188B-2 and images were taken for cell growth. Scale bar indicates 50 μm. (D) Cells treated with siFAM188B and stained by Hoechst33342. White arrowheads: fragmented nuclei. Scale bar indicates 20 μm. (E) Effect of siFAM188B on cell cycle was measured by flow cytometry. (F) Cells were stained with Annexin V/PI at 72 hr after siRNA transfection and analyzed by flow cytometry (PI stained cells apoptosis population: UR and UL).

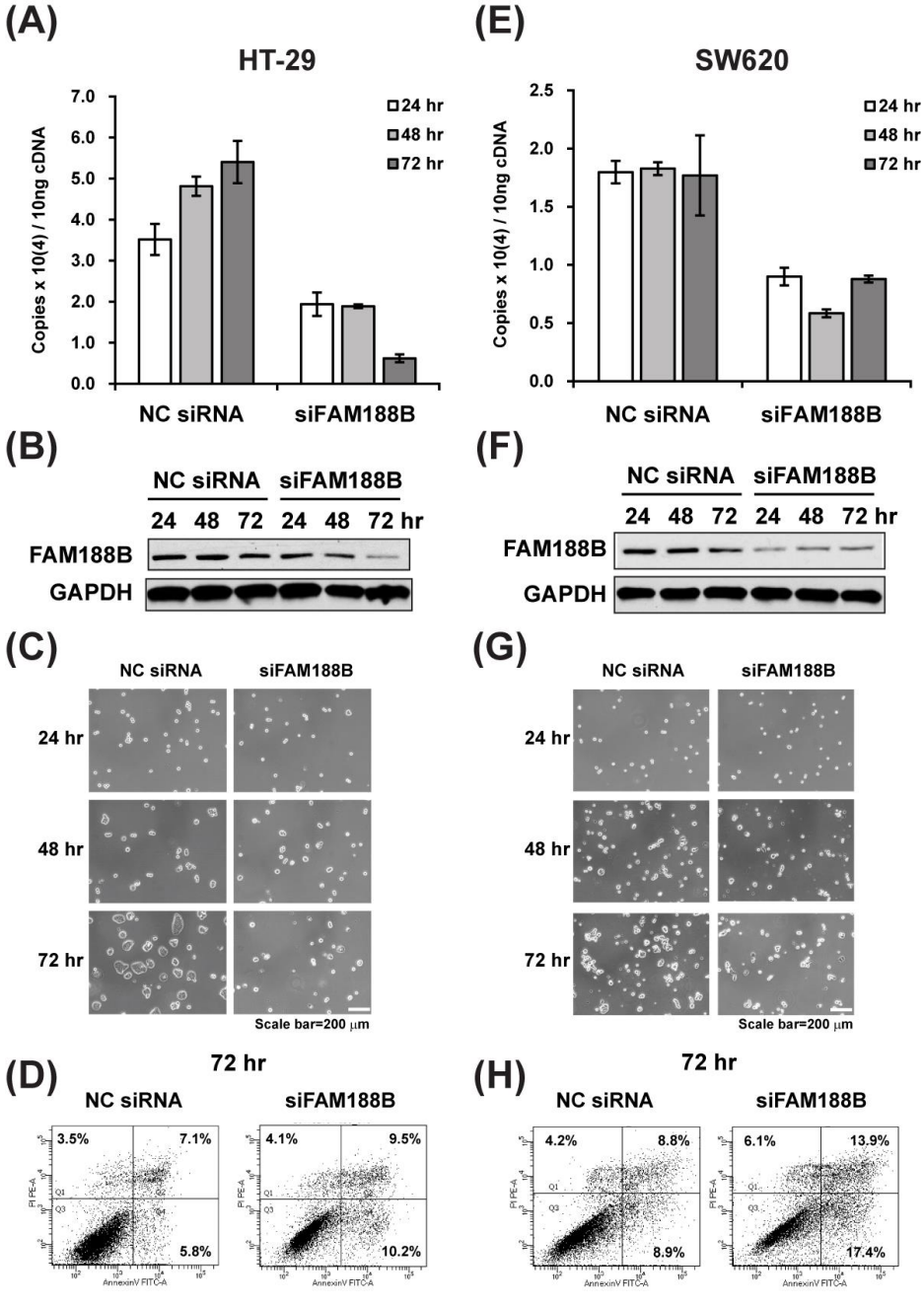


Figure 16. Confirmation of the cell death phenotype in HT-29 and SW620 colon cancer cell lines treated with siFAM188B. (A and E) mRNA level of *FAM188B* on HT-29 cells (A) or SW620 (E) treated with NC siRNA or siFAM188B was absolutely quantitated by qRT-PCR. Error bar indicates S.D. (B and F) Lysates of HT-29 (B) or SW620 cells (F) were separated by SDS-PAGE and detected by anti-FAM188B antibody and anti-GAPDH antibody as an internal control. (C and G) The images of siFAM188B treated HT-29 (C) and SW620 cells (G) were captured for 72 hr. Scale bar represents 200 μm. (D and H) HT-29 (D) or SW620 cells (H) were stained with Annexin V/PI at 72 hr after siRNA transfection and analyzed by flow cytometry (PI stained cells apoptosis population: UR and UL).

13. The identification of FAM188B-interacting partners

To investigate the mechanism of FAM188B involvement in cell death, potential cellular FAM188B binding partners were analyzed by LC/MS-MS (Figure 17A). Mass peaks from MS searches against the database identified a total of 104 unique proteins bound to FAM188B. These proteins were analyzed using the DAVID server[41] and String-DB[42] to cluster proteins according to their involvement in intracellular biological processes and evidence-based interacting groups, respectively. As expected, based on results obtained by FAM188B down-regulation, many FAM188B-binding proteins were involved in cell cycle regulation and apoptosis. Notably, clusters on the interaction map of FAM188B-binding proteins revealed several clusters including the tumor-suppressor proteins p53 as well as USP7 (Figure 17B, arrows). Interestingly, when I immunoprecipitated p53 proteins, I observed that FAM188B as well as USP7 were in the p53 immunocomplexes (Figure 17C), indicating that FAM188B forms a complex with p53.

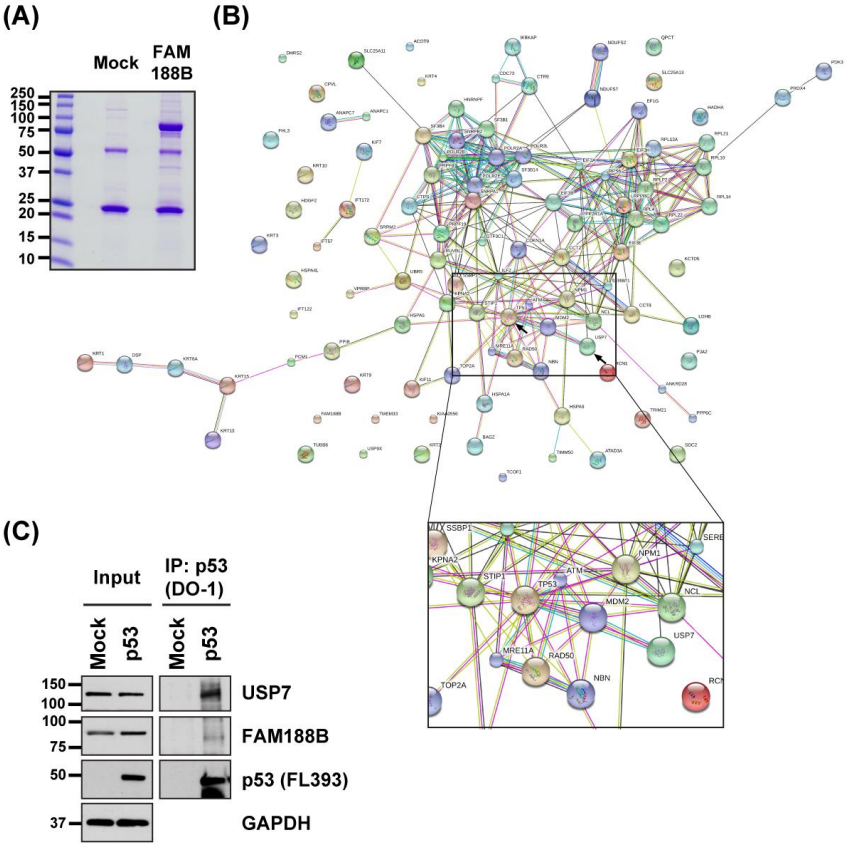
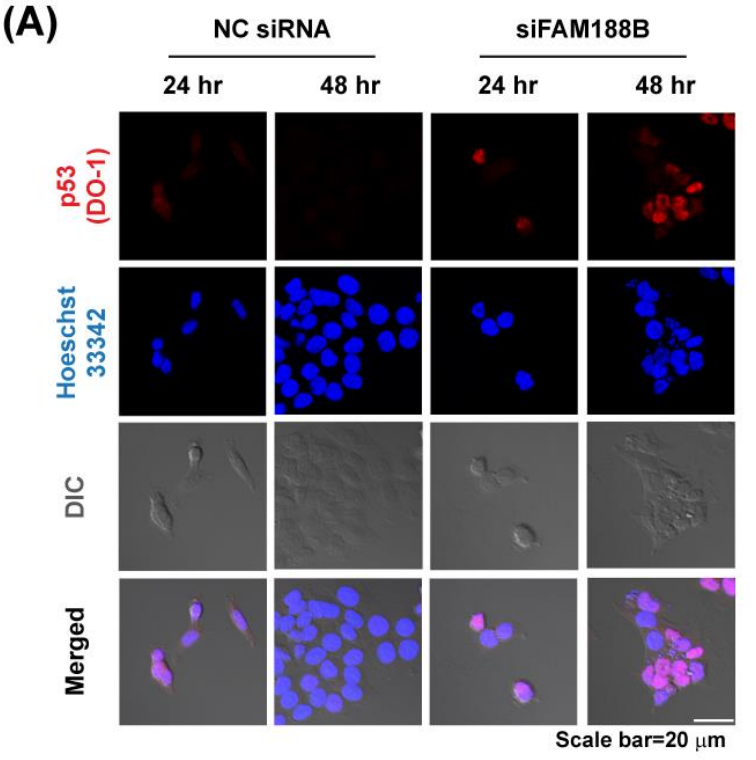


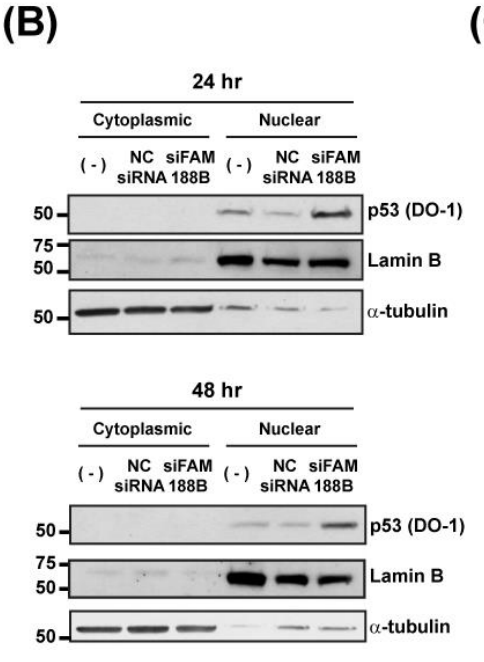
Figure 17. Analysis of FAM188B-interacting proteins. (A) FLAG-tagged FAM188B was overexpressed in HEK-293 cells and immunoprecipitated complexes of FLAG-tagged FAM188B were resolved on SDS-PAGE gels. Image of Coomassie-blue staining of the gel is shown. (B) The resolved proteins in (A) were processed for LC/MS-MS analysis and searched against String-DB to see the interactions in 'Evidence View'. Black arrow indicates interacting protein separately confirmed by immunoprecipitation. (C) Validation of FAM188B interaction to USP7 and p53 by immunoprecipitation of HEK-293 cell lysate with p53 (DO-1) antibody and immunoblotted with indicated antibodies.

14. FAM188B silencing activates p53 and its downstream pathway.

Because p53 is an important regulator for cell death control, I further examined a possible association of p53 in the siFAM188B-induced apoptosis in more detail. When cells were stained for p53, more p53 were localized in the nucleus in the siFAM188-treated cells than in the NC siRNA-treated cells (Figure 18A). Consistent with these immunocytochemistry data, more p53 protein was detected in the nucleus fraction than in the cytosolic fraction when FAM188B was knockdown (Figure 18B). Next, I tested whether p53 is activated in the FAM188B knocked down cells by immunoblotting using antibodies that recognize Ser15-phosphorylated p53 (active form of p53).[43] FAM188B knockdown increased p53 as well as Ser15-phosphorylated p53, and thus enhanced protein levels of p53-regulated genes, including p21, PUMA, and BAX, which are in the apoptosis pathway (Figure 18C).

To further test p53 activation transcriptionally, promoter assay was performed using p21 promoter-luciferase construct because p21 is known to be a p53 target gene.[44] siFAM188B treatment increased the transcriptional activity of p53 toward its downstream target p21 by ~ 2-fold (Figure 19A and 19B). Moreover, chromatin immunoprecipitation (ChIP) assays using p53 antibodies showed increased binding of p53 to BAX (Figure 19C) and PUMA promoters (Figure 19D) in the siFAM188B-treated cells. These results show that FAM188B down-regulation activates p53, resulting in upregulation of apoptosis related genes, including BAX and PUMA, which leads to cell death.





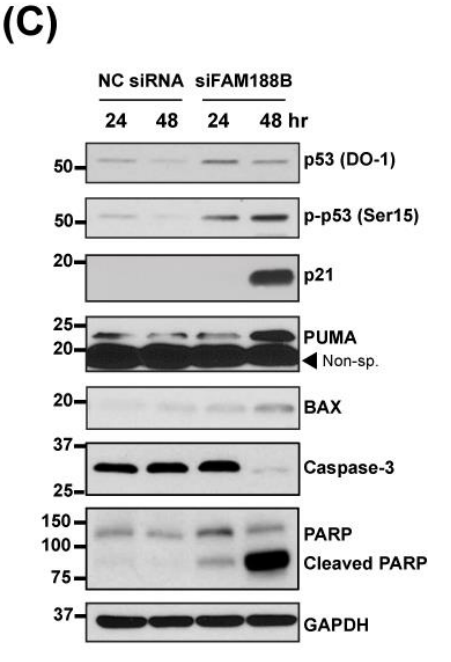


Figure 18. Knockdown of FAM188B activates the p53 downstream pathway. (A) p53 was primarily stained by p53 (DO-1) antibody and AlexaFlour 594 was used as secondary antibody. Images were obtained by fluorescence inverted microscope. (Scale bar = 20 μm) (B) translocated p53 was detected by Western blot after cytoplasmic and nuclear fraction. PARP and α-tubulin are the nuclear and cytoplasmic control, respectively. (C) activated p53 downstream pathway proteins in HCT-116 treated with NC siRNA or siFAM188B were detected by indicated antibodies.

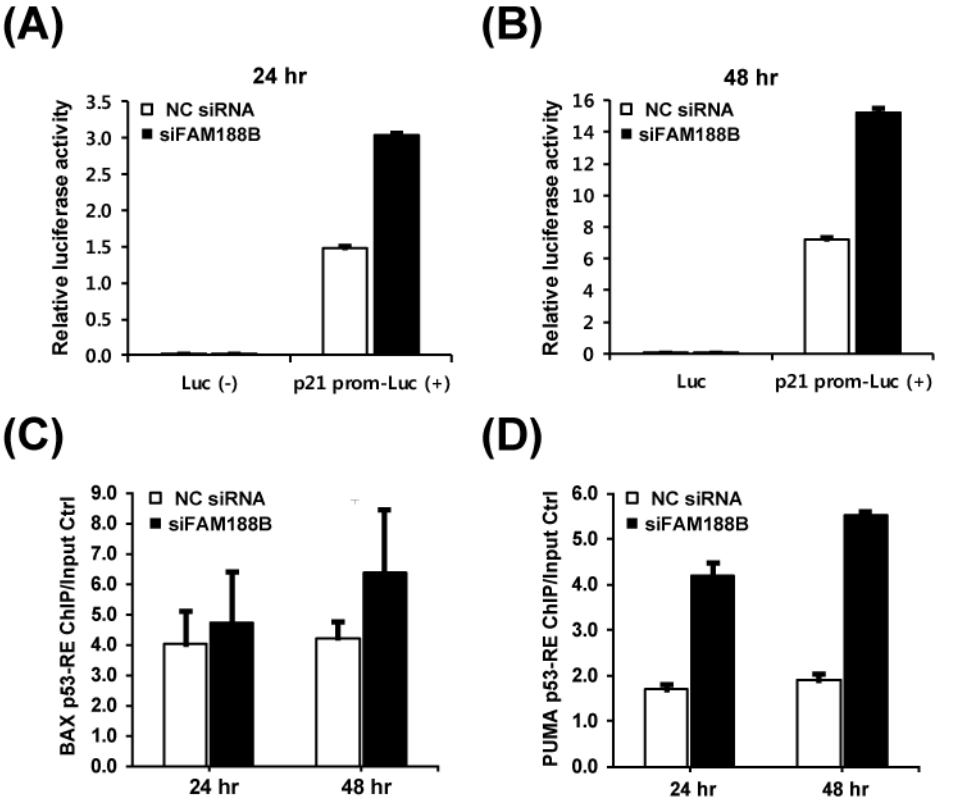


Figure 19. Knockdown of *FAM188B* results in activation of the p53 downstream pathway. (A-B) Activation of the p21 promoter was significantly increased by siFAM188B treatment at 24 (A) and 48 hr (B). (C-D) The p53-responsive element of BAX (C) and PUMA promoter regions (D) was measured by ChIP qRT-PCR assays.

15. Expectation of FAM188B three-dimensional protein structure

From the FAM188B amino acid sequences, I converted it as a pdb file format in Phyre2 web server[45] and visualized using PyMOL 1.7 program. The structure looked like a "golf club head" (Figure 20). Unexpectedly, FAM188B has many alpha helix structures, indicated by cyan, which easily crosses the biological membranes because of its hydrophobicity. The predicted sites of USP7 binding motifs, indicated by yellow, were located in surface region. Therefore, I hypothesized the interaction between FAM188B and USP7 regulates p53.

To compare the 3D-modeling structure, another web server SWISS-MODEL[46] was used. However, SWISS-MODEL showed only 5 homologous protein structure parts such as FGFR1 Oncogene Partner (FOP), FAM63A/MINDY-1, Probable GTPase engC, Noggin, and PlyCB. FOP was identified a fusion partner of FGFR1 in leukemia-associated chromosomal translocation and found as a centrosome protein.[47] FAM63A/MINDY-1 is recently identified as a deubiquitinase specifically cleaving at Lys-48-linked poly ubiquitin which is a protein degradation signal.[11] engC is a ribosome GTPase protein in *Streptococcus* genus. Noggin is known to regulate a major class of metabologens, bone morphogenetic proteins (BMPs).[48] PlyCB is a lysin from the streptococcal bacteriophage C_1 subunit B.[49] After the analysis of these proteins annotation, structural comparisons give no information for the function of FAM188B.

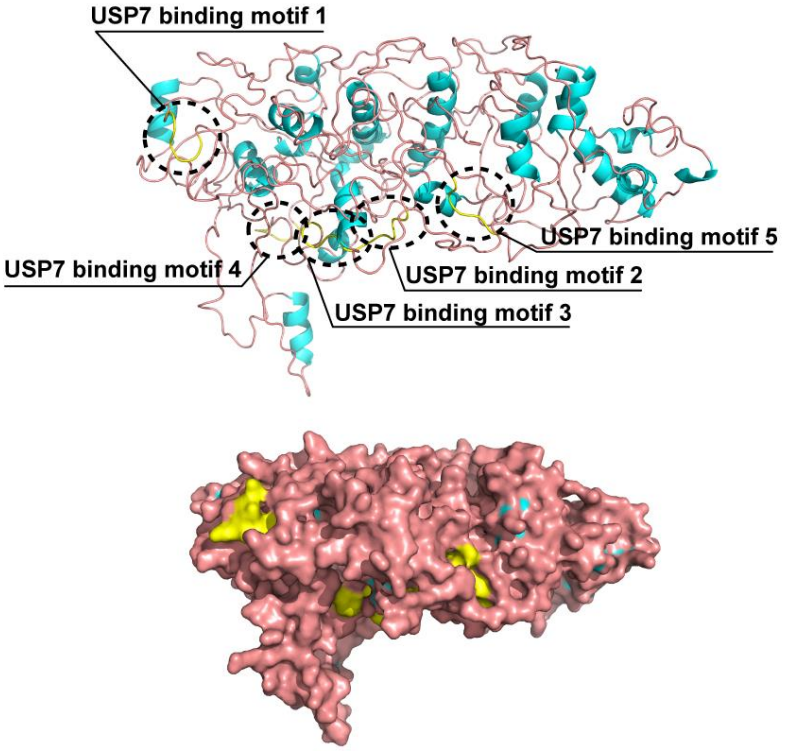


Figure 20. Prediction of FAM188B ternary structure. Predicted FAM188B structure was drawn by ribbon diagram (upper) and surface representation (lower). Cyan represents alpha helix structure. Yellow indicates the USP7 binding motifs.

16. FAM188B regulates p53 stability *via* interaction with USP7

To determine how FAM188B knockdown enhances p53 protein levels, FAM188B-interacting proteins were identified by LC/MS-MS (Figure 17B). Out of FAM188B binding proteins, USP7 and p53 related to apoptosis. I hypothesized that USP7 is a mediator to regulate deubiquitination of p53. In accordance with this, the online protein domain prediction site, 'Eukaryotic Linear Motif resources',[50] also predicted the presence of a USP7 binding motifs in FAM188B (Figure 21A). USP7 protein was detected in FAM188B immunoprecipitates in the cells with FAM188B over-expression (Figure 21B), indicating a complex formation of FAM188B with USP7. To investigate which motif of FAM188B is responsible for its complex formation with USP7, either FLAG-tagged wild type or mutant FAM188B with deletion of USP7 binding motif-1 (ΔUSP7-1) or USP7 binding motif-2 (ΔUSP7-2) were overexpressed in HCT-116 cells, followed by immunoprecipitation of exogenous FAM188B using anti-FLAG antibodies. USP7 was consistently found in the wild type FAM188B immunocomplexes but USP7 levels decreased in the mutant FAM188B immunocomplexes (ΔUSP7-2), suggesting that USP7-2 motifs of FAM188B are important for its complex formation with USP7 (Figure 21C). Next, to examine whether USP7 interacts with p53, Co-IP assay was performed after etoposide treatment to increase p53 expression. Etoposide treatment resulted in an increase in p53 and a decrease in FAM188B. p53 was found in the USP7 immunocomplexes and this complex formation increased in etoposide

treatment (Figure 21D). Next, I tested whether FAM188B levels affect to complex formation of USP7 and p53. A basal level of USP7 was detected in the p53 immunocomplexes. However, interestingly, this complex formation appeared to increase in the siFAM188B treated cells after 24 hr, and it became weaker at 48 hr although the endogenous USP7 protein expression was not changed by FAM188B silencing (Figure 21E).

To confirm whether FAM188B is involved in p53 deubiquitination, I examined the change in level of ubiquitinated p53 after FAM188B knockdown in the cells expressing HA-tagged ubiquitin. Compared with NC siRNA-treated cells, lower p53 level was detected in the HA-immunoprecipitated complexes from siFAM188B-treated cells (Figure 22A). In addition, the level of HA-tagged ubiquitin decreased in the p53 immunocomplexes from siFAM188B-treated cells compared with those of NC siRNA-treated cells. A lower level of endogenous ubiquitinated p53 was also detected in the p53 immunocomplexes from siFAM188B-treated cells than those of NC siRNA-treated cells (Figure 22B), while ubiquitination of total protein was increased in siFAM188B-treated cells.

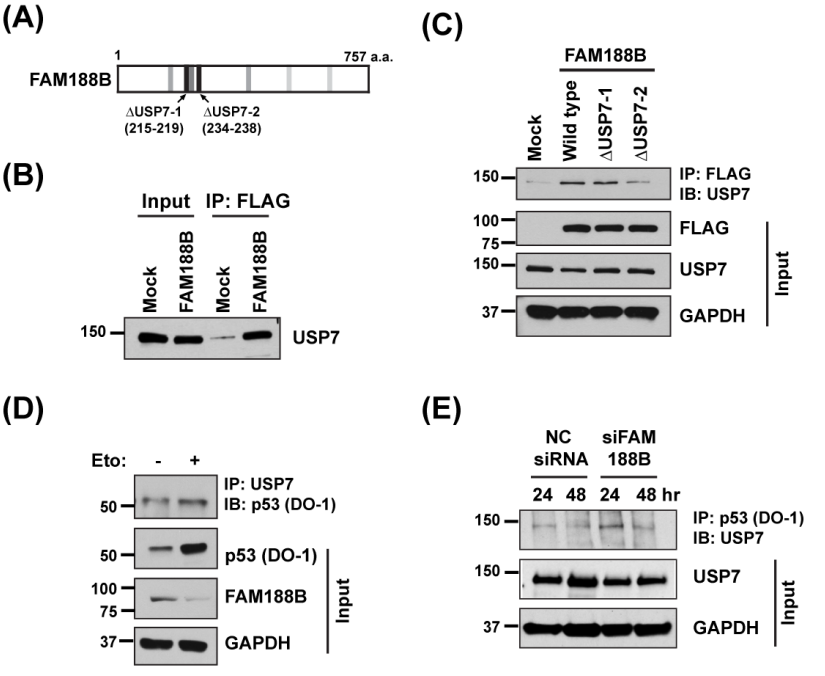


Figure 21. USP7 interacts with FAM188B. (A) Predicted position of seven USP7 binding motifs was indicated on FAM188B protein. ΔUSP7-1 and -2 are the position of deletion mutants. (B) Co-IP of overexpressed FAM188B and USP7 in HCT-116 cell. (C) Verification of the interaction between FAM188B and USP7 using overexpression of wild type and ΔUSP7 FAM188B constructs. (D) Lysates from HCT-116 cells treated with or without etoposide (5 μM) were immunoprecipitated and immunoblotted with indicated antibodies. Eto: etoposide (E) FAM188B silencing increased the interaction between USP7 and p53. Cell lysates from HCT-116 cells treated with NC siRNA or siFAM188B for 24 and 48 hr were immunoprecipitated with anti-p53 antibody and immunoblotted with anti-USP7 antibody.

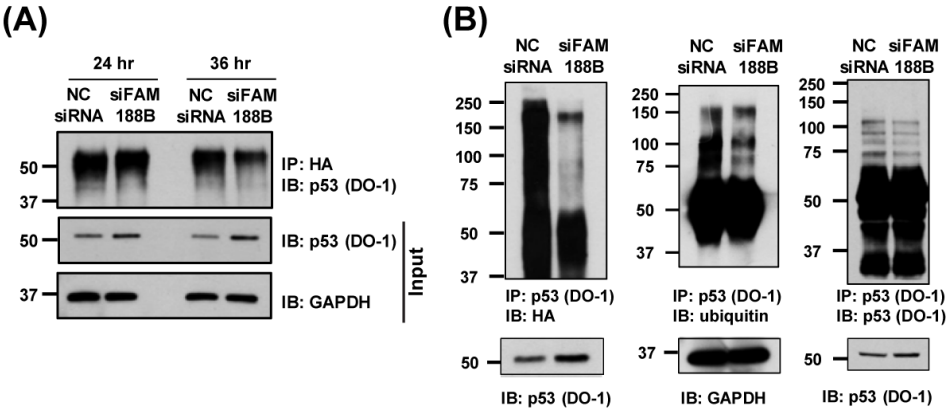


Figure 22. Knockdown of FAM188B regulates p53 deubiquitination. (A) HA-tagged ubiquitin (5 μg) was transfected to HCT-116. Cell lysates from HCT-116 treated with NC siRNA or siFAM188B were immunoprecipitated by anti-HA antibody and immunoblotted with indicated antibodies. (B) Cell lysates from HCT-116 co-transfected with HA-tagged ubiquitin and NC siRNA or siFAM188B were immunoprecipitated by anti-p53 (DO-1) antibody and immunoblotted with indicated antibodies.

17. FAM188B knockdown inhibits tumor growth *in vivo*.

To further investigate the effect of FAM188B down-regulation on tumor growth, I established a shFAM188B-inducibile HCT116 stable cell line. Doxycycline induced FAM188B shRNA, which led to FAM188B down-regulation (Figure 23A). The numbers of colonies were reduced by FAM188B shRNAs especially in shFAM188B#2 stable cell line (Figure 23B). Next, I examined the effects of FAM188B knockdown on anchorage-independent colony formation by soft agar assay. When HCT-116 cells were treated with siFAM188B, anchorage-independent colony formation was significantly decreased (Figure 23C). These data indicated that FAM188B might have an oncogenic function.

To test the effect of FAM188B silencing on tumor growth *in vivo*, HCT-116 cells were subcutaneously xenografted into BALB/c nude mice, and FAM188B siRNA was delivered by electroporation. Tumor volume was regularly measured after treatment with FAM188B siRNA. The growth of siFAM188B-treated tumors was significantly reduced from the first week compared to that of NC siRNA treated tumors (Figure 24A, top). When the tumors were removed from the sacrificed mice, siFAM188B-treated tumors were smaller than the NC siRNA-treated tumors (Figure 24A, bottom). To verify the FAM188B knockdown in the xenografted tumors, tumor tissues were processed for immunostaining using anti-FAM188B antibodies. Immunohistochemistry analysis showed that FAM188B protein levels reduced,

while p53 protein levels increased, in the siFAM188B-treated tumors (Figure 24B). These data indicate that FAM188B expression is important for tumor growth *in vitro* and *in vivo*.

Taken altogether, our data indicate that FAM188B has a critical oncogenic effect, possibly *via* enhancing p53 ubiquitination and thus p53 down-regulation. Therefore, targeting FAM188B could be a good strategy to control tumor growth.

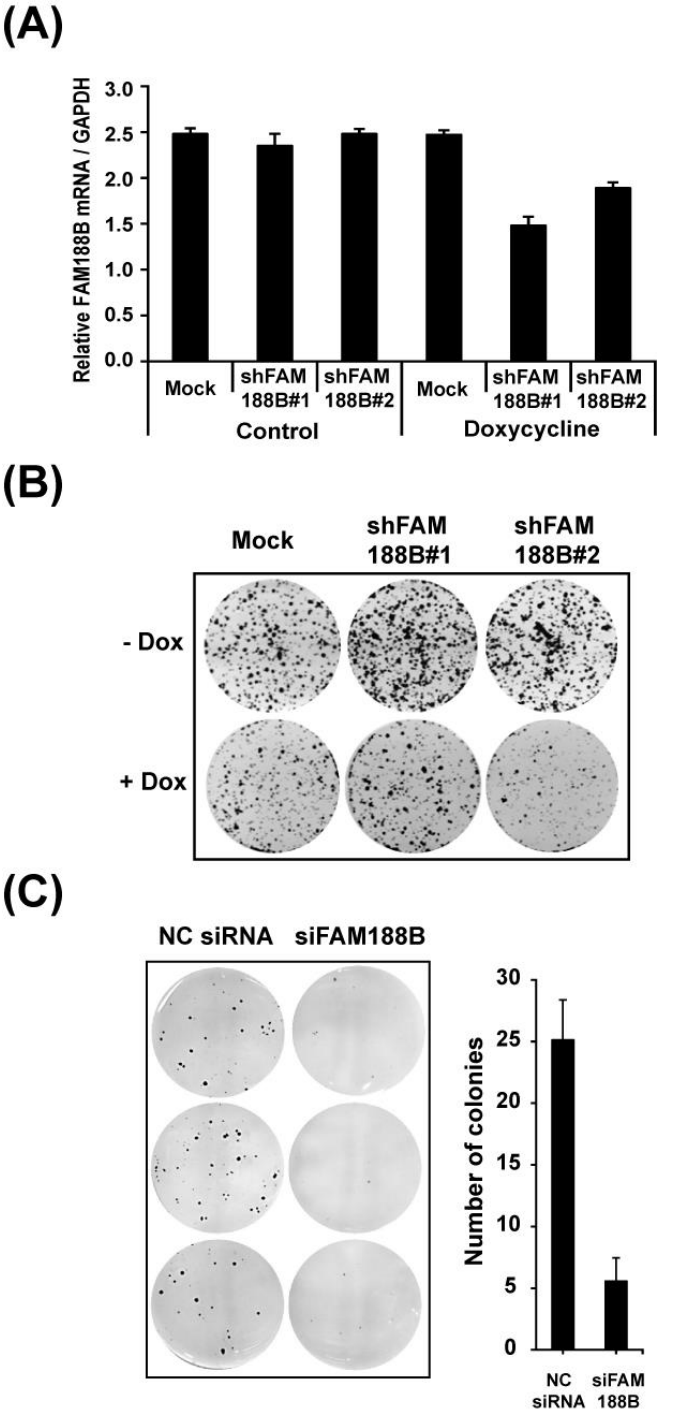
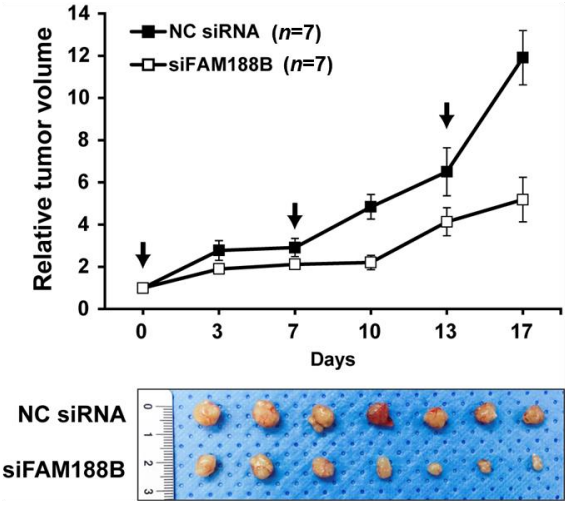


Figure 23. FAM188B knockdown effects on cell proliferation. (A) shRNA stable cell lines were confirmed by quantitative real-time PCR. mRNA expression of *FAM188B* was normalized by *GAPDH*. Data were shown as mean±S.D. (B) FAM188B downregulated cells were inhibited proliferation in HCT-116 as indicated with colony forming assay. DOX indicates doxycycline. (C) HCT-116 cells (5.0×10^3) treated with NC siRNA or siFAM188B was cultured in soft agar for 18 days. The colonies were stained with INT solution. All experiments were performed triplicate.

(A)



(B)

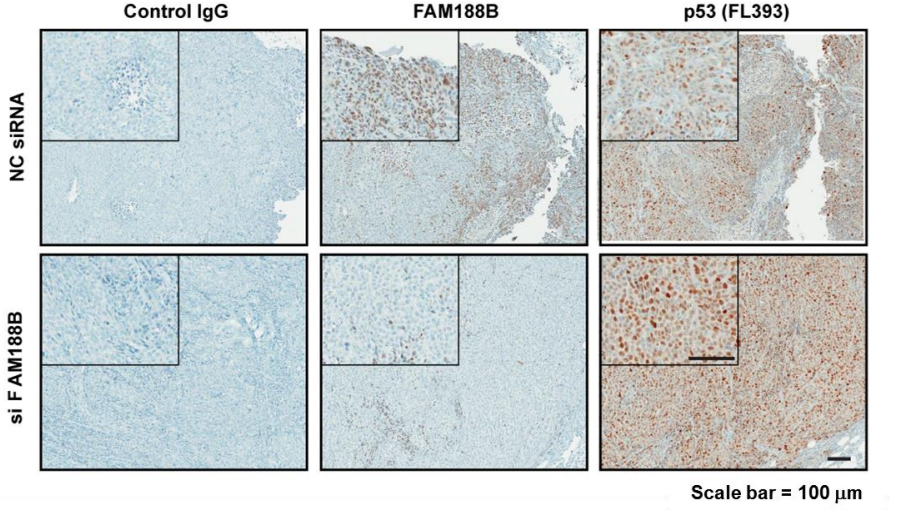


Figure 24. Knockdown of FAM188B inhibits cell proliferation *in vivo*. (A) HCT-116 cells (2.0×10^6) were injected into the nude mice (*n*=7) subcutaneously and tumor growth was monitored twice a week. When tumor size reached $30mm^3$, siRNAs were treated a week for 3 wk. Black arrow indicates the siRNA treatment. Tumor size was measured at indicated time points (representative images of tumors; NC siRNA (top lane) and siFAM188B (bottom lane)). (B) Immunohistochemistry of FAM188B and p53 protein detected from tumor tissues from mice using control IgG, anti-FAM188B and anti-p53 (FL393) antibodies (Scale bar = 100 μm).

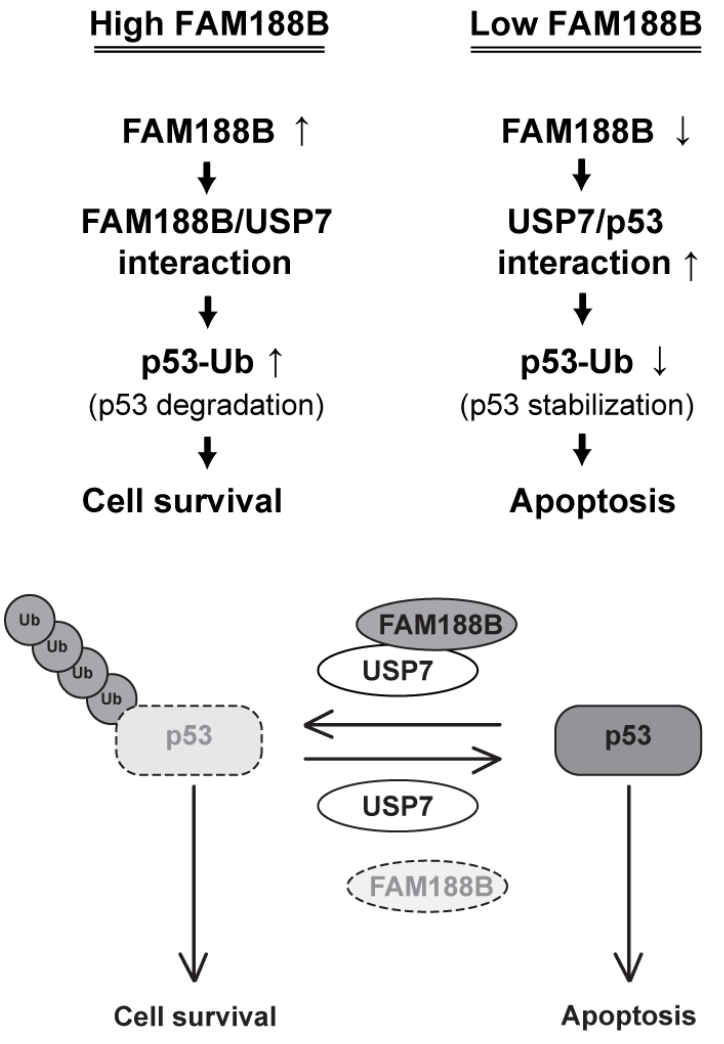


Figure 25. A proposed model for regulation of p53 stability by FAM188B and USP7. High level of FAM188B maintains the tumor suppressor protein p53 as low level through interacting with USP7 to prevent the deubiquitination of p53 (left, upper). Reduced FAM188B stabilizes the p53 which interacts with free USP7 and results in cell death (right, upper). FAM188B might be act as an oncogene in this proposed model (lower).

IV. DISCUSSION

Cancer progression is the result of various biological processes, and may also be altered by unexplored functions of novel/hypothetical genes, and/or variants of known genes. Despite the tremendous effort devoted to analyze available genomic information, as much as 59% of human genes were annotated as 'hypothetical' when the human genome was first reported,[8, 51] and 24–31% of Entrez/ensemble database entries are currently annotated as 'uncharacterized'.[10] FAM188B was also annotated as 'a hypothetical protein' for which the transcript is the only supporting evidence for functionality in public databases when this study was begun. Hypothetical proteins are increasing because of the development of the sequencing techniques. The database in UniProt is also increasing with accumulation of the sequencing data and there is a huge difference between UniProtKB/TrEMBL which is automatically annotated and is not reviewed and UniProt/Swiss-Prot which is manually annotated and is reviewed,[52] suggesting that unannotated novel proteins are dramatically identified every year. In addition to these accumulating data, up-regulated hypothetical proteins have been discovered in cancers.[53] Therefore, identifying the characteristics of hypothetical protein is important for understanding mechanisms of tumorigenesis as well as provision of novel therapeutic targets.

To determine whether FAM188B is a protein-coding gene or a non-coding gene, I verified poly-(A) tail which is a marker of mature mRNA. If the gene does encode a protein, it would be interesting to characterize the effect of the

protein on tumorigenesis; otherwise, the only possible use of the gene would be as a biomarker. However, the mature mRNA sequence of FAM188B from the colon cancer cell line HCT-116 showed a poly-A signal sequence in its 3' UTR, suggesting that this gene could indeed encode a protein. This evidence led us to generate a rabbit polyclonal antibody to detect the FAM188B protein. Using the generated anti-FAM188B specific antibody, I could detect over-expressed or endogenous FAM188B protein by immunoblotting and immunohistochemistry. From the results, the location of FAM188B is in the cytosol and rarely exists in nucleus. Furthermore, as revealed by transcriptome profiles of the public databases including the CCLE, Oncomine and NCBI GEO,[54] the expression of FAM188B was significantly enhanced in tumors at the mRNA level. These results suggest that FAM188B has important housekeeping functions in maintaining cell viability.

Generally, the first way to try to understand the function of specific genes is to remove it. FAM188B siRNA was treated to gastric cancer cell line AGS and/or colon cancer cell line HCT-116 and I observed retarded proliferation and suspended dead cells. It suggests FAM188B is an important gene for survival. However, identifying genuine interacting partners—a key to the known world of biological pathways—is crucial to understand the working mechanisms of this unknown protein. The catalogue of FAM188B-interacting proteins was defined through immunoprecipitation of FAM188B followed by LC/MS-MS analysis. A clustering of these proteins according to their Gene Ontology (GO)

biological process showed that the major clusters were ‘protein translation’ and ‘cell death/apoptosis,’ followed by ‘chromatin regulation’ and ‘RNA-binding proteins’. Among these biological processes, cell death and protein degradation categories were of interest, because knockdown experiments suggested that this protein might be involved in regulating cell survival. Thus, I verified the FAM188B-interacting proteins identified by mass-spectrometry using co-immunoprecipitation, and then proceeded to characterize FAM188B functions related to cell death. Immunoprecipitation using an anti-p53 (DO-1) antibody confirmed the interaction of FAM188B with p53. Intriguingly, GO analyses categorized several proteins into an ‘ubiquitin-dependent protein’ group; among them was USP7 (also known as HAUSP), which clustered with p53 as a FAM188B-interacting protein. USP7 is known to stabilize p53 by deubiquitinating it, as well as its inhibitor MDM2.[55, 56.] As such, USP7 has come to be considered a therapeutic target in many cancers.[14, 21] In breast cancer, TSPYL5 was reported to reduce p53 levels through physical interactions with USP7.[57] In addition to USP7, USP2a, and USP10 are also involved in regulating p53 ubiquitination in different contexts;[14] thus, evidence supporting USP7 as the major p53 modulator is inconclusive. In our study, however, the amount of FAM188B-bound USP7 dramatically increased upon FAM188B overexpression in HCT-116 cells. Conversely, FAM188B down-regulation increased unbound USP7 and this free-USP7 may decrease the level of ubiquitinated p53. The fact that USP7 plays a pivotal role in deubiquitinating both p53 and MDM2, as

noted above, seemingly undermines the interpretation that USP7 is a p53 stabilizer. This may be the reason why the decrease of ubiquitinated p53 in siFAM188B treated cells was not extreme. However, the shorter half-life of MDM2 compared to p53, as a result of MDM2 self-ubiquitination,[22, 58] may ultimately lead to cell death through accumulated p53. If a mediator exists, that could be the answer to the questions "What determines whether p53 or MDM2 is the primary USP7 substrate?"[19] and "What determines how USP7 is regulated?".[22] FAM188B might be the mediator. In tumor cells, high levels of FAM188B would be predicted to function by eliminating p53 and keeping it at a low level to allow tumor cells to grow. Therefore, elevated expression of FAM188B in tumor tissues indicates that FAM188B would strongly inhibit p53 deubiquitination and thereby provide tolerance against stressful conditions, such as treatment with cytotoxic agents.

The most intriguing result in this study was confirming the tumorigenicity of FAM188B. The colony forming ability of FAM188B was significantly abolished by silencing its expression *in vitro*. Interestingly, in soft agar colony formation assays, a golden standard to determine cellular transformation and tumorigenicity,[59] FAM188B silencing showed dramatic reduction of anchorage-independent colony growth. Moreover, *in vivo* tumor growth assays also revealed that FAM188B led to a dramatic difference in tumor masses of xenografted HCT-116 in BALB/c nude mice. Accordingly immunohistochemical analyses revealed that FAM188B is a vital gene for

proliferation or survival. Although additional studies with human tissue are also required to assess feasibility, these results suggest that FAM188B over-expression could be used as a predictive biomarker for cancer diagnosis, and inhibiting FAM188B activity could be utilized as the putative target of cancer progression. Although further studies are required to reveal how FAM188B expression is regulated, our findings clearly support the conclusion that FAM188B is an important regulator of p53 stability in growing cells.

As a mediator of USP7, I have elucidated mechanisms that affect cell survival by controlling ubiquitination of p53, but there are many things to be revealed. First, I have to prove that FAM188B is actually acting as deubiquitinase. In recent study,[11] FAM188B was classified as MINDY family and named probable ubiquitin carboxyl-terminal hydrolase MINDY-4, but failed to provide experimental evidence for its function.

During the additional experiment to stratify unknown functions of FAM188B in the tumor cells, I also observed that down-regulation of FAM188B results in decrease of FOXM1 expression which is a transcription factor and promotes oncogenesis with its aberrant upregulation. Since knockdown of FOXM1 sequentially reduced the CD44 expression level,[60] I speculate that FAM188B promotes the tumorigenesis through the FOXM1-CD44 pathway independently of the ability to regulate the stability of p53 through USP7. As a result, I observed the elevated expression of CD44v8-10, one of the CD44 variants and elucidated as a marker of cancer

stem cell (CSC).[61]

In this study, the hypothetical protein FAM188B was revealed to be a genuine protein whose expression is significantly elevated in colon cancer cell lines. On the basis of FAM188B loss-of-function analyses, I suggest that FAM188B functions to sustain cell viability by decreasing p53 activation, through inhibition of the deubiquitinase USP7. This provides significant insight into the importance of FAM188B in enhancing cell survival, as well its use as a potential target in cancer therapy. Further research on the details of FAM188B regulatory mechanisms, as well conditions with p53 mutations, will increase our understanding of FAM188B as a potential therapeutic target.

V. CONCLUSION

In summary, I experimentally proved that hypothetical protein FAM188B is a genuine protein and evolutionarily conserved from fish to human. I identified FAM188B downregulation leads to cell death, suggesting that it is an important gene for survival. I also found that reduction of FAM188B enhances chemotherapeutic effects in gastric cancer *in vitro* and *in vivo*. To understand the mechanism of FAM188B, HCT-116, a colon cancer cell, was used and found that the p53 pathway was activated by FAM188B knockdown. I investigated ubiquitination to determine how the p53 pathway is activated since USP7 and p53 interact with FAM188B. Finally, I identified FAM188B regulates p53 stability through interacting with deubiquitinase USP7 *in vitro*. Taken together, a putative oncogene FAM188B interacts with USP7 and regulates p53 stability by participating in deubiquitination. Our study of FAM188B will contribute to the knowledge on its mechanism in tumorigenesis and provide a clue for the development of future cancer therapeutics.

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ABSTRACT (IN KOREAN)

위장관암에서 hypothetical protein FAM188B의 분자세포생물학적 기능 분석

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위암에서 엑손(Exon)이 다르게 발현하는 유전자들을 분석한 이전 연구에서, FAM188B는 염기 서열 분석을 통하여 유전자라고 추정되지만, 실험적으로는 증명되지 않은 “가설단백질 (hypothetical protein)”이었다. 가설단백질에 대해서는 현재까지 많은 연구가 되지 않았고, FAM188B의 발현 여부 및 그 기능에 대해서 밝혀진 바가 없었다. 이번 연구에서는 인간의 FAM188B 아미노산 서열 정보를 다른 종과 비교 분석하여 FAM188B가 척추동물들에서 진화적으로 보존되어 있는 것을 확인하고, 실제로 mRNA 및 단백질이 발현하는

것을 증명하였다. FAM188B가 진행성 위암으로 진행하는데 관여하는 것을 mRNA 분석을 통해 추정하였다. 종양 형성에 있어 FAM188B의 기능과 메커니즘을 밝히기 위해 위암 및 대장암 세포주에 FAM188B siRNA를 처리하여, 성장 억제와 세포가 사멸되는 현상을 관찰하였다. 세포 사멸은 FAM188B의 감소에 의해 p53이 증가하고 활성화되어 유도되었다. Mass spectrometry를 이용하여 FAM188B는 p53과 탈유비퀴틴화 효소인 USP7과 상호작용한다는 것을 발견하였다. 세포 및 동물 실험에서 FAM188B의 발현을 감소시키면 p53과 복합체 형성뿐만 아니라 p53의 유비퀴틴화를 전반적으로 감소시키며, 이 결과는 FAM188B와 USP7의 결합이 약해져 자유로워진 USP7에 의한 p53 탈유비퀴틴화가 진행된다는 증거를 제시하였다.

종합하면 FAM188B는 탈유비퀴틴화 효소인 UPS7과의 상호작용을 통하여 p53의 안정성을 조절하여 세포 성장을 증진시키며, 또한 FAM188B는 종양유전자로 추정할 수 있다. 따라서 앞으로 FAM188B 발현을 조절한다면 종양 성장을 억제하는 치료제로서의 새로운 가능성이 있다는 것을 제시하였다.

핵심되는 말: FAM188B, 탈유비퀴틴화 효소, USP7, p53, 가설단백질, 암

PUBLICATION LIST

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