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The mechanism study of LAMB1 as diagnostic and prognostic biomarker for gastric cancer

조선대학교 대학원

글로벌바이오융합학과

이 하 나

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조선대학교 대학원

글로벌바이오융합학과

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지도교수 김 석 준

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조선대학교 대학원

글로벌바이오융합학과

이 하 나

이하나의 석사학위논문을 인준함

위원장 조선대학교 교 수 전 홍 성 (인)

위 원 조선대학교 교 수 김 정 수 (인)

위 원 조선대학교 교 수 김 석 준 (인)

2020년 11월

조선대학교 대학원

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국 문 초 록

위암의 진단 및 예후 바이오마커로써 LAMB1의 기전연구

이 하 나
지도교수 : 김 석 준
글로벌바이오융합학과
조선대학교 대학원

위암은 전세계에서 5번째로 높은 암 발병률을 보이며, 특히 동북아시아 특이적으로 발생하는 호발암이다. 그 중, 우리나라의 위암 발병률은 동북아시아의 다른 국가에 비해 가장 높은 수준이다. 현재 위암의 치료 방법으로 외과적 수술, 면역요법, 항암화학요법, 방사선 치료 등 다양한 방법들이 존재한다. 최근 2018년 보건복지부 통계에 의하면, 위암은 조기 치료시 5년 생존률이 약 90% 이상의 높은 생존률을 보이지만 2기 위암환자의 5년 생존률은 약 75%, 3기는 약 40%, 4기 약 5%의 생존률을 보인다. 이러한 통계수치는 위암이 말기로 진행이 될수록 생존률이 급격하게 줄어들며 위암의 조기 발견 및 조기 치료에 대한 중요성을 의미한다. 특히, 우리나라 위암 환자의 5년 생존률은 75.8%로 미국의 32.1%에 비해 높게 보고되고 있지만, 아직까지 위암의 진단 및 치료제 개발에 대한 연구결과는 미미한 수준이다. 따라서 위암의 진단, 예후 및 치료적 접근이 가능한 바이오마커 개발이 필요한 실정이다.

본 연구는 질병 진단, 예후 및 치료 접근성이 높은 분자적 위암 바이오마커 발굴 및 발굴된 분자의 기능을 검증하고자 하였다. 효과적인 연구를 진행하기 위해 기존에 보고된 위암환자의 public GEO 데이터를 이용하여, 분자적 바이오마커를 선별하였으며, 역전사 중합효소연쇄반응, 웨스턴 블랏, 증식, 침윤, 이동성 분석 등 다양한 분자기전 실험 방법을 본 연구에 이용하였다.

먼저 위암환자의 public GEO 데이터들을 이용하여 GEO2R 분석을 통해 기존의 위암의

바이오마커로 알려진 인자를 제외한 위암환자에서 발현이 높은 유전자를 발굴하였고 그 중 위암의 분자적 바이오마커의 가능성을 보인 Laminin β1(LAMB1)을 선별하였다. 추가적으로 웨스턴 블랏을 통해 유전자 발현뿐만 아니라 LAMB1의 단백질 발현이 위암환자의 조직에서도 증가되어 있음을 확인하였다. 예후인자로써의 가능성도 확인하기 위해 Km plot 분석을 통해 위암환자에서 LAMB1의 과발현은 환자의 생존률에 좋지 않은 예후를 미치는 것을 확인하였다. 특히, 기존 보고를 통해 간암에서 LAMB1은 세포 조립에 관여하여 세포의 침윤 및 전이에 역할을 가지는 것으로 알려져 있지만, LAMB1이 위암의 증식 및 전이에 미치는 기전연구는 아직 보고된 것이 없어, LAMB1이 위암에서 증식과 전이에 미치는 기전을 확인하기 위해 먼저 위암 세포에서 간섭 RNA를 이용하여 LAMB1의 발현을 억제하여 위암세포의 증식 및 콜로니 형성의 감소와 더불어 침윤과 이동이 억제됨을 확인할 수 있었다. 반대로 LAMB1의 발현을 증가시켰을 경우에는 이러한 현상이 증가함을 알 수 있었다. 결과적으로 LAMB1은 위암의 증식 및 전이에 영향을 끼치는 것을 증명하였다. 기존에 보고된 자료를 통해 MAPK/ERK 활성이 LAMB1의 발현에 영향을 끼치는 것을 확인하여, 위암세포에 ERK 억제제 U0126를 처리한 결과 웨스턴 블랏을 이용하여 LAMB1의 발현이 저해되었음을 확인하였고, 추가로 세포의 증식, 침윤, 이동 또한 감소함을 확인할 수 있었다. 그리고 LAMB1의 전사를 조절하는 전사인자를 확인하고자 예측프로그램을 이용하여 LAMB1의 promoter에 결합할 가능성이 높은 전사인자를 선별하여 위암세포에 간섭 RNA 이용하여 LAMB1의 유전자 발현을 확인한 결과, ERK 하부인자로 알려진 c-Jun이 LAMB1의 유전자 발현을 조절하는 것을 확인하였고, 추가적으로 염색질 면역침전을 통해 c-Jun이 LAMB1의 프로모터에 직접 결합하여 LAMB1의 발현을 조절함을 확인하였다.

본 연구의 결과들을 통해 LAMB1은 위암환자의 조직에서 정상 조직보다 높은 발현을 가지며, LAMB1의 과발현은 위암환자의 생존률에 좋지 않은 예후를 가지는 것을 확인하였다. 특히, LAMB1은 ERK/c-Jun 신호기전에 의해 위암의 증식 및 전이를 조절하는 것도 확인하였다. 결과적으로 LAMB1은 위암의 증식 및 전이를 조절하는 분자적 바이오마커로 가능성이 있을 것으로 생각되며, 이를 조절하여 향후 위암의 진단 및 예후에 사용될 수 있으리라 기대한다.

I. INTRODUCTION

Cancer is considered a leading cause of death in the world, it known to count of 9.6 million deaths in 2018 for all deaths [1]. The deaths of cancer are expected to 12 million deaths in 2030 [2]. And the cancer of global burden was showed the six cancer, including lung, breast, colorectal, prostate, liver and gastric cancers [3]. Gastric cancer is the most common burden of malignant tumor and important of mortality rate in worldwide [4, 5]. According to the recently data, new cases of approximately 1,000,000 gastric cancer are occurred worldwide [6]. Despite advances in cancer therapy of radiation therapy, including of surgery, chemotherapy and radiation therapy, but, the 5-year relative survival rate is remains poor percentage of approximately 20% [7]. The early stage of gastric cancer therapy showed that the 5-year survival rate about 90% [8]. But, only about approximately 20% of gastric cancers are found in early stage. Therefore, it is important and necessary to explore biomarker to provide effective prognosis and new therapies of gastric cancer.

Biomarker is cellular detectors (DNA, RNA, protein, peptide and metabolites) of the physiological characteristic and is used to identify a normal or abnormal condition [9, 10]. Biomarker for gastric cancer is the diagnosis markers such as the carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 125, SLE, BCA-225 [11]. However, the measurement of biomarker in cancer patients serum is not insufficient detection due to the limit of sensitivity in the gastric cancer detection. Therefore, it is important to discover efficiently detective biomarker of gastric cancer.

The LAMB1, also called a Laminin β1, is composed number of approximately 1,700 amino acid and existed in most tissues [12]. Generally, the Laminin β has a role of initiated cell assembly. The function of cell assembly plays important role in the cancerous cell invasion and metastasis [13]. And mRNA expression of LAMB1 is showed highly expression in several invasive cancer [14]. The extracellular matrix (ECM) is a various function of tumor microenvironment, including biochemical and biomechanical effects of cancer development, progression, metastasis and immune function [15, 16]. So the upregulated protein expression of ECM components promote cell proliferation, invasion and

migration in cancer [17]. The ECM has a composition containing representatively collagens, glycoproteins and proteoglycans [18, 19]. Laminin is representative contained of ECM glycoproteins and is composition of large heterotrimeric protein complex, containing laminin subunit alpha, beta and gamma (also called the LAMA, LAMB and LAMC) which can assemble at least 16 laminin isoforms [20]. Recently, the LAMA, LAMB and LAMC are related to effect of oncogenic function and potential as a therapeutic target marker by using data analysis [21-24]. In hepatocellular carcinoma (HCC), expression of LAMB1 promotes tumor progression at the invasion through PDGF/La axis-mediated LAMB1 translation [25]. And LAMB1 is upregulated in serum of colorectal cancer patients and can be a potential serological biomarker for diagnosis of coloreactal cancer [26]. However, the biological function and mechanism of LAMB1 remain undefined in gastric cancer. Thus, in this study was focused on study aim to explore whether effect of LAMB1 in gastric cancer.

The mitogen-activated protein kinase (MAPK) pathway-is component to JNK, ERK and p38 pathway-can promote dysregulation of cell cycle, induced abnormal cell growth, and is essential for invasion and differentiation [27, 28]. ERK pathway is a major trigger for the development of most cancers. In several studies, laminin is involved in MAPK/ERK signaling pathways regulated LAMB1 expression in HCC [25, 29]. The c-Jun is known to compose complex of AP-1, has function of transcription factors to activate or suppress target gene transcription [30]. Also, c-Jun is the oncogenic transcription factor in most cancer. The excessive expression of c-Jun mediates the various biological function of proliferation, invasion and migration [31-33]. ERK pathway regulates c-Jun [34]. The ERK-dependent c-Jun/AP-1-is involved cell invasion in human osteosarcoma cells [35]. And c-Jun increases the expression of matrix metalloproteinase-1 (MMP1), the collagen component [36]. In addition, c-Jun regulates laminin 1 which increases cell growth [37]. c-Jun is regulated by ERK pathway. So, ERK and c-Jun are associated with regulation of ECM component-induced cell growth and motility in cancer.

In this study showed that LAMB1 was upregulated and related to survival in public GSE datasets of gastric cancer patients. And LAMB1 promoted the biological function of growth and motility in gastric cancer cells. In addition, LAMB1 was regulated by ERK/c-Jun signaling pathway. Take the results showed that the LAMB1 through ERK/c-Jun pathway could serve as a potential target to biomarker and therapeutic target of gastric cancer.

II. MATERIALS AND METHODS

II-1. Cell lines and Cell culture

The human gastric cancer cell lines AGS, MKN-28, YCC-2, SNU-216, SNU-601 and SNU-668 were purchased from the Korea Cell Line Bank. Human normal gastric epithelial cell lines (GES-1) was obtained Yonsei Cancer Center. All cells were cultured in RPMI-1640 medium (Welgene, Korea) containing 5% fetal bovine serum (Corning Costar, USA) and 1% antibiotic-antimycotic (Gibco, USA) in a 37°C incubator in an atmosphere of 5% CO_2.

II-2. Patient tissues

The human tissues of gastric cancer patients were obtained National Cancer Center (NCC). A total of six pairs of gastric cancer tissues were used for detection of LAMB1 expression by using western blotting.

II-3. Lentivirus infection and transient transfection

LAMB1 cDNA clones was purchased from Korea Human Gene Bank (KHGB). Human LAMB1 construct was cloned into pCMV-3Tag-1A plasmid in the *BamH1/Xho1* restriction enzyme sites, to generate the pCMV-LAMB1. Primers for LAMB1 with *BamH1/Xho1* enzyme site; 5′-AATACGGATCCATGGGGCTTCAGTTGCT-3′ (forward, contained *BamH1* enzyme site) and 5′-ACGAGCTCGAGTTACAAGCATGTGCTATACA-3′ (reverse, contained *Xho1* enzyme site). The cells were transfected using Lipofectamine 2000 (Invitrogen) for plasmid DNA infection. AGS, MKN-28, SNU-601 and SNU-668 cells were transfected with 2 μg of plasmid DNA (empty vector and pCMV-LAMB1) using 6.25 μg Lipofectamin 2000 at 60-mm cell culture dish. After incubation of 48 h, cells were washed with cold 1×PBS and used RNA or protein isolation. The sequences of human siRNAs purchased

from Genolution Inc., The siRNA sequences used in this study were as follows: LAMB1_siRNA#1, 5′-GAGAUAACCUUCUGGAUUCUU-3′ (forward) and 5′-GAAUCCAGAAGGUUAUUAUCUCUU-3′ (reverse); LAMB1_siRNA#2, 5′-GGAUUUCUACCAUGAUUUAUU-3′ (forward) and 5′-UAAAUCAUGGUAGAUUUAUU-3′ (reverse); c-Jun_siRNA#1, 5′-GAGCUGGAGCGCCUGAUAAUU-3′ (forward) and 5′-UUAUCAGGCGCUCCAGCUCUU-3′ (reverse); c-Jun_siRNA#2, 5′-GAGCGGACCUUAUGGCUACUU-3′ (forward) and 5′-GUAGCCAUAAGGUCCGCUCUU-3′ (reverse) ; C/EBPβ_siRNA, 5′-CCAAGAAGACCGUGGACAAUU-3′ (forward) and 5′-UACUCGGCCGGCUUCUUGCUU-3′ (reverse); RXRα_siRNA, 5′-GGGAGAAGGUCUAUGCGUCUU-3′ (forward) and 5′-GACGCAUAGACCUUCUCCCUU-3′ (reverse); XBP-1_siRNA, 5′-GGUAUUGACUCUUCAGAUUUU-3′ (forward) and 5′-AAUCUGAAGAGUCAAUACCUU-3′ (reverse); YY1_siRNA, 5′-GGAUAACUCGGCCAUGAGAUU-3′ (forward) and 5′-UCUCAUGGCCGAGUUAUCCUU-3′ (reverse). The cells were transfected using Lipofectamine RNAiMAX (Invitrogen) for siRNA transfection. AGS and MKN-28 cells were transfected with 20 μM of LAMB1_siRNA#1, #2 or 30 μM of siRNA (c-Jun, C/EBPβ, RXRα, XBP-1, YY1) using 6.25 μg Lipofectamin RNAiMAX at 60-mm cell culture dish. After incubation of 48 h, cells were washed with cold 1×PBS and used RNA or protein isolation.

II-4. Total RNA isolation and Reverse transcriptase-polymerase chain reaction (RT-PCR)

The total RNA was isolated using RNAiso Plus reagent (TaKaRa Bio, Japan). The cells were added with 1 mL of RNAiso Plus reagent, and added 200 μL of chloroform. After reaction for 5 min at RT, each sample was centrifuged at 13,200 rpm for 15 min at 4℃. Then, the clear supernatant was transferred to new 1.5 mL EP-tube and added to 2-propanol. After reaction for 5 min at RT, each sample was centrifuged at 13,200 rpm for 10 min at 4℃. The supernatant was removed and added to 500 μL 75% ethanol. And each sample was centrifuged at 13,200 rpm for 5 min at 4℃. After removing supernatant, each sample was added to diethyl pyrocarbonate (DEPC)-treated water for dissolve of

isolated RNA. And cDNA was synthesized by using cDNA Master Mix (ToYoBo, Japan). Then, PCR was performed by using HiPi Plus 5×PCR Master Mix (ELPIS-BIOTECH, Korea). The primer sequences used in this study were as follows: LAMB1, 5′ -AGGTTGGAGCTGCCTCAGTA-3′ (forward) and 5′-ACACTCCCTGGAAACAGTGG-3′ (reverse); c-Jun, 5′-TGACTGCAAAGATGGAAACG-3′ (forward) and 5′ -CCGTTGCTGGACTGGATTAT-3′ (reverse); CEBPB, 5′-CAAGAAGCCGGCCGAGTAC-3′ (forward) and 5′-TTGTCCACGGTCTTCTTGGC-3′ (reverse); RXRA, 5′ -CCTTTCTCGGTCATCAGCTC-3′ (forward) and 5′-TGTCAATCAGGCAGTCCTTG-3′ (reverse); XBP1, 5′-AATCGAGGAAGCACCTCTCA-3′ (forward) and 5′ -AAGCATCCAGTAGGCAGGAA-3′ (reverse); YY1, 5′-GGATAACTCGGCCATGAGAA-3′ (forward) and 5′-GGTTGTTTTTGGCCTTAGCA-3′ (reverse); GAPDH, 5′ -TGCACCACCAACTGCTTAG-3′ (forward) and 5′-GGATGCAGGGATGATGTTC-3′ (reverse). PCR products were used for 1% agarose gel electrophoresis with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology). GAPDH was used as the control.

II-5. Protein preparation and western blotting

The protein were extracted using RIPA buffer (Biosesang Inc., Korea) containing phosphatase and protease inhibitor solution (GeneDEPOT, USA). After incubation at 4℃ for 35 min, each sample was centrifuged at 13,200 rpm at 4℃ for 25 min. The protein concentration was measured by the bovine serum albumin (BSA) Protein Assay (Thermo Fischer Scientific, USA). The protein sample was resolved to 10% - 8% SDS-PAGE gels and transferred to PVDF membranes. And each membrane was blocked using 5% skim milk in 1×PBS buffer with 0.05% Tween-20 (PBST). Each membrane was incubated for overnight at 4℃ on rocking shaker with primary antibody diluted 1:1000 in 5% BSA in PBST buffer. Then, Each membrane was followed by exposure to secondary antibody diluted 1:5000 for 1 h 30 min at RT. Band was visualized by chemiluminescence (Bio-Rad, Korea).

II-6. Antibodies

The monoclonal antibodies used were against anti-laminin β-1, anti-ERK, anti-p-ERK, anti-β-actin (Santa Cruz Biotechnology). The polyclonal antibodies were against anti-c-Jun (Santa Cruz Biotechnology), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Bioworld Technology, USA). The GAPDH was used to loading control in western blotting.

II-7. Colony formation assay

The transfected cells were incubated for 24 h, cells were seeded in the 35-mm cell culture dish (1,500 cells). During incubation, medium was changed every 2-3 days. After 10 days of incubation, colonies were washed with cold 1×PBS and fixed with 1% glutaraldehyde for 10 min. And colonies were stained with 0.5% crystal violet for 10 min at RT on rocking shaker. Then, colonies that had formed in each dish were counted. Each experiment was performed in triplicate experiments.

II-8. Transwell invasion and migration assay

The cells were transfected with vector or siRNA, and incubated for 24 h. Transfected cells were seeded in FBS-free medium at 2×10^4 cells on upper chamber (Corning Costar), on filter coated with Matrigel (BD Biosciences, Korea) for invasion assay or filter coated with 0.5 mg/mL collagen type I (BD Biosciences, Korea) for migration assay. The low chamber were added to 1 mL of RPMI-1640 medium containing 10% FBS and 1% antibiotics, and followed by incubation for 20 h at a 37℃ incubator in an atmosphere of 5% CO_2. Invading and migrating cells were quantified after H&E staining and counted in each well using wide-field microscopy.

II-9. Cell cycle analysis

The cells were transfected with siRNA, and incubated for 48 h. After incubation, cells

were washed with 1×PBS and fixed to 5 mL of 75% ethanol overnight at -20℃. After fixation, cells were washed twice with cold 1×PBS and dispersed in a staining solution containing 50 μg/mL RNase A and 50 μg/mL PI solution in PBS for 15 min at RT. Cell cycle distribution was measured by PI staining using a CytoFLEX flow cytometer (Beckman Coulter, USA).

II-10. Chromatin immunoprecipitation (ChIP) assay

AGS and MKN-28 cells were cultured in 150-mm culture dish (2×10^5 cells). After 2 days of incubation, medium contained cells were treated with 1% formaldehyde for cross-linking. The cells were incubated at RT for 10 min. ChIP assay was performed using Pierce Agarose ChIP Kit (Thermo Fisher Scientific), according to the manufacture's instructions. After cell pellet isolation, the cell pellets were treated with lysis buffer 1 for membrane lysis. The sample was centrifuged at 9000 ×g for 3 min at 4℃ and the supernatant was discarded. And the sample was added to buffer contained micrococcal nuclease (MNase) and incubated in 37℃ water bath for 15 min. The sample was centrifuged at 9000 ×g for 5 min at 4℃ and the supernatant was discarded. The sample was treated to lysis buffer 2 for nucleic membrane lysis and incubated on ice for 15 min. The sample was centrifuged at 9000 ×g for 5 min and the supernatant of digested chromatin was transferred to new 1.5 mL tube. Then, 10% total supernatant were used as input samples. The remained supernatant were subjected to immunoprecipitation using c-Jun antibody for overnight at 4℃ on rocking platform. After overnight, the supernatant contained c-Jun antibody were added ChIP Grade Protein A/G Plus Agarose for 1 h at 4℃ on rocking platform. Then, the supernatant were washed three times IP Wash Buffer. The elution buffer was added to washed samples and treated proteinase K for remove the protein in samples. And, input sample was treated proteinase K. The samples of elution and input were transferred to DNA clean-up column. And clear DNA was obtained. The antibodies used in this study were as follows; anti-c-Jun (Santa Cruz Biotechnology) and normal rabbit immunoglobulin G (lgG) were used to immunoprecipitated DNA containing complexes. Anti-c-Jun was the same that antibody used in western blotting. Anti-rabbit lgG

was contained this ChIP kit and used as negative control. Primers were designed with LAMB1 promoter binding sites; Primer 1, 5′-CTTCTCTGGGCCTTATTTCG-3′ (forward) and 5′-CTGCTACCCTTAGCAATGGA-3′ (reverse), which amplified a 204-bp region; Primer 2, 5′-GGAGAATCGTCGAGATGAGC-3′ (forward) and 5′-CTGGGCAACAAGAGCAAAAC-3′ (reverse), which amplified a 213-bp region. And PCR was performed by using HiPi Plus 5×PCR Master Mix.

II-11. Data accession and analysis

The public GSE data had been deposited in NCBI's Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo) and generated using GEO Series accession number GSE2685, GSE13861, GSE33651 and GSE63089. These data were normalized by using GEO2R. *P*-value less than 0.05 was considered statistically significant.

II-12. Gene ontology (GO) annotation and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID; david.ncifcrf.gov) and generated using 56 genes of merged four GSE data. *P*-value less than 0.05 was considered to indicate a statistically significant difference.

II-13. Survival analysis

Survival analysis were performed for overall survival (OS), first progression (FP) and post progression survival (PPS) of gastric cancer patients (https://kmplot.com/analysis) and generated using gene symbol LAMB1 (Affy ID: 201505_at and 211651_s_at).

II-14. Statistical analysis

Analysis of data was performed using GraphPad Prism5 software (GraphPad, USA). Statistical analyses were analyzed using the Student's t-test. A *P*-value less than 0.05 was considered statistically significant. And all data presented as mean ± SEM.

III. RESULTS

III-1. LAMB1 is identified in public GEO datasets for gastric cancer

To identify the genes that was upregulated in gastric cancer, i used public database of four GEO datasets (GSE2685, GSE13861, GSE33651 and GSE63089) to analyze the DEG. Then, i searched that genes were upregulated in gastric cancer tissues compared to normal tissues and were classified at each public microarray data. The overall process of analysis was showed in Figure 1. And our used methods and detail information of GEO datasets were described in Table 1. GEO2R analysis of public GEO datasets showed that venn diagrams of 56 genes merged in four GSE data were overexpressed in gastric cancer tissues (Figure 2). To determine into biological pathway of these 56 genes, i performed analysis of Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. GO annotation and KEGG pathway enrichment analysis showed that differentially upregulation of genes was composed various pathway. These results were associated with extracellular matrix organization, ECM interaction and structural constituent (Figure 3.A and B). ECM is important for initiate cancer development and cancer growth. In addition, ECM is detected in cancer patient tissues and serums. LAMB1 was associated with the involvement of the extracellular matrix signaling, including ECM-receptor interaction, focal adhesion, extracellular matrix organization and structural constituent (Table 2 and 3). The data showed that LAMB1 is relate the extracellular matrix signaling and adhension, is mediated cell motility in cancer. Therefore, i further explored LAMB1. And, to identify distribution of overall upregulation gene expression in gastric cancer tissue compared to normal tissues, i showed that LAMB1 locus was presented to upregulation gene position in gastric cancer tissues (Figure 4). These results indicated that i performed screening of upregulated genes using gastric cancer patient microarray data and LAMB1 was selected for further research.

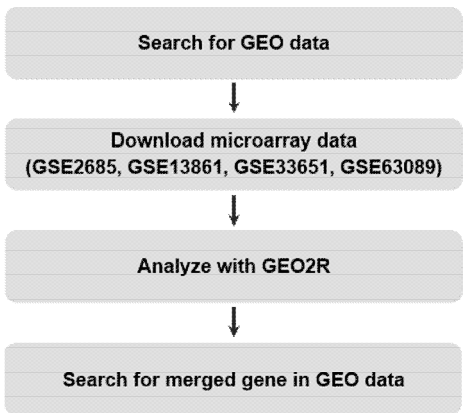

Figure 1. The microarray data is performed to search upregulated genes in gastric cancer. GSE data of gastric cancer was searched (GSE2685, GSE13861, GSE33651 and GSE63089). And, public GSE data was analyzed by using GEO2R, so, upregulated genes were identified in gastric cancer.

Table 1. The relevant information gastric cancer datasets of GSE microarray

GEO accession	Contributors, year	Country	Platform	Total samples	Cancer tissues	Normal tissues
GSE2685	Hippo Y et al., 2002	Japan	GPL80	30	22	8
GSE13861	Cho J et al., 2011	USA	GPL6884	90	71	19
GSE33651	Park D et al., 2011	South Korea	GPL2895	52	40	12
GSE63089	Zhang X et al., 2014	China	GPL5175	90	45	45

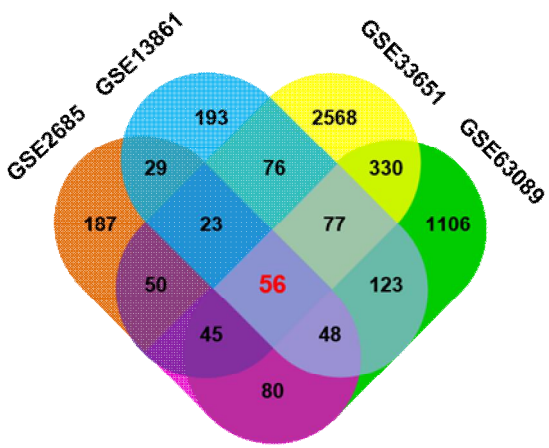

Figure 2. The upregulated genes are showed DEGs in GSE data from gastric cancer. The upregulated genes (logFC > 0.7, logFC > 0 means gene overexpression in gastric cancer tissues) in gastric cancer tissues were indicated by venn diagram. The red number showed the results for merged genes in four public GEO datasets.

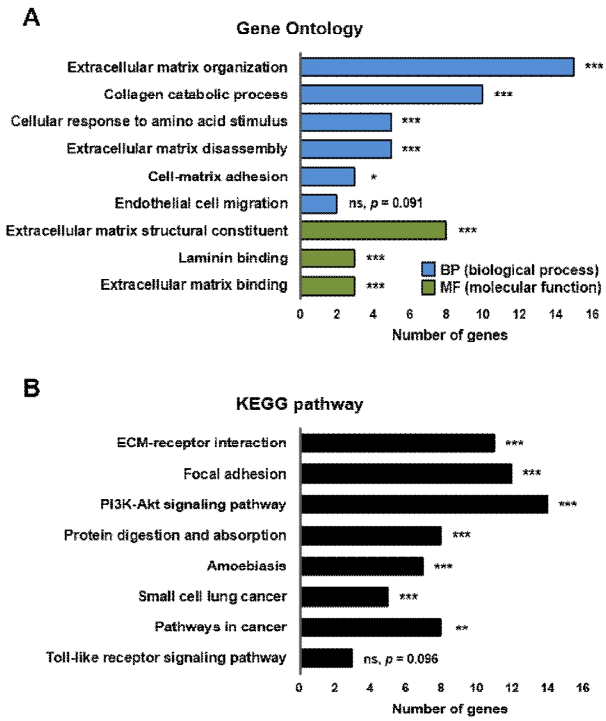

Figure 3. GO annotation and KEGG pathway enrichment analysis of 56 upregulated genes. Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the DAVID. (**A**) Gene Ontology analysis of biological process and molecular function for upregulated genes in gastric cancer. (**B**) KEGG pathway analysis for upregulated genes in gastric cancer. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns: not significant.

Table 2. Enriched Gene Ontology (GO) identified in upregulated differentially expressed genes

Gene Ontology	Gene count	P-value	Gene symbol
BP (biological process)			
Extracellular matrix organization	15	1.5E-15	COL1A1, COLA2, COL3A1, COL4A1, COL4A2, COL5A2, COL6A3, ITGB1, **LAMB1**, LUM, NID2, SPP1, SPARC, SERPINB5, THBS1
Collagen catabolic process	10	5.2E-13	CTSK, COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A2, COL6A3, MMP1, MMP7
Cellular response to amino acid stimulus	5	1.7E-05	COL1A1, COL1A2, COL3A1, COL4A1, COL5A2
Extracellular matrix disassembly	5	1.1E-04	BMP1, CTSK, MMP1, MMP7, SPP1
Cell-matrix adhesion	3	3.5E-02	COL3A1, ITGB1, NID2
Endothelial cell migration	2	9.1E-02	FAP, STAT1
MF (molecular function)			
Extracellular matrix structural constituent	8	1.5E-09	COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A2, **LAMB1**, LUM
Laminin binding	3	2.8E-03	LGALS1, ITGB1, THBS1
Extracellular matrix binding	3	3.0E-03	CTSK, ITGB1, THBS1

Table 3. Enriched KEGG pathway identified in upregulated differentially expressed genes

KEGG pathway	Gene count	P-value	Gene symbol
ECM-receptor interaction	11	6.30E-12	COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A2, COL6A3, ITGB1, **LAMB1**, SSP1, THBS1
Focal adhension	12	2.10E-09	COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A2, COL6A3, ITGB1, CCND2, **LAMB1**, SPP1, THBS1
PI3K-Akt signaling pathway	14	3.60E-09	COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A2, COL6A3, CCND2, CCNE1, HSP90AB1, ITGB1, **LAMB1**, SPP1, THBS1
Protein digestion and absorption	8	1.80E-07	ATP1B3, COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A2, COL6A3
Amoebiasis	7	1.10E-05	COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A2, **LAMB1**
Small cell lung cancer	5	7.60E-04	COL4A1, COL4A2, CCNE1, ITGB1, **LAMB1**
Pathways in cancer	8	2.60E-03	COL4A1, COL4A2, CCNE1, HSP90AB1, ITGB1, **LAMB1**, MMP1, STAT1
Toll-like receptor signaling pathway	3	9.60E-02	CTSK, SPP1, STAT1

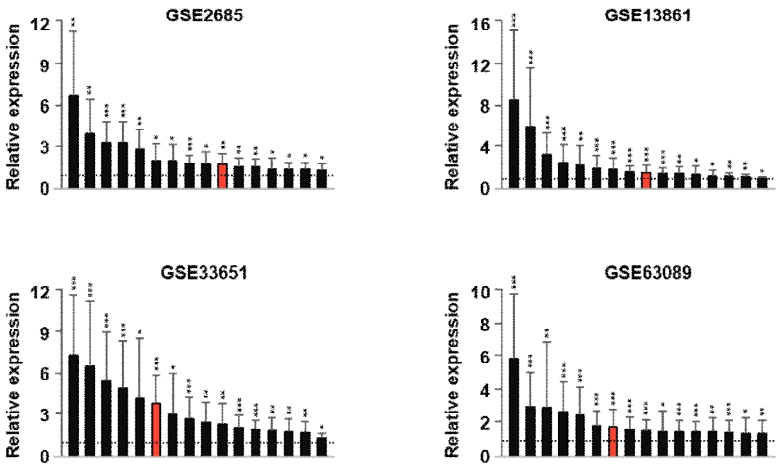

Figure 4. The relative expression of upregulated genes in GSE data of gastric cancer. The overall upregulated genes expression distribution in gastric cancer compared to normal were indicated by histograms. The red bar showed the loci of LAMB1 expression in GEO datasets of gastric cancer. Presenting of all data as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

III-2. LAMB1 is significantly upregulated and correlated with risk of poor prognosis in gastric cancer

The mRNA expression of LAMB1 in used public GEO datasets for gastric cancer patients was confirmed to significantly the upregulation in gastric cancer (Figure 5). And, to identify the LAMB1 expression in gastric cancer patient tissues, i performed in six sets patients of normal tissues and tumor tissues patient for gastric cancer the western blotting. The LAMB1 expression was significantly upregulated in tumor tissues compared to normal tissues in 4 of 6 gastric cancer patients (Figure 6). To identify the survival effect of LAMB1 in gastric cancer patients, i used the online public resource of 201505_at and 211651_s_at by survival analysis. Analysis of survival rate showed that the LAMB1 was associated with poor overall survival (OS), first progression (FP) and post progression survival (PPS) in high expression of gastric cancer patients compared to low expression of patients (Figure 7). These results observed that LAMB1 is upregulated in gastric cancer and associated with poor prognosis in gastric cancer patients.

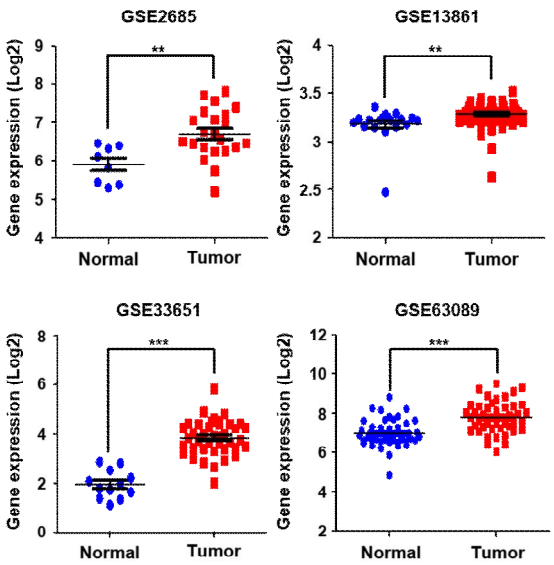

Figure 5. LAMB1 is significantly upregulated in gastric cancer. The results showed that LAMB1 expression was overexpressed in gastric cancer tissue from public database of GEO datasets. Presenting of all data as mean ± SEM. $^{**}P < 0.01$; $^{***}P < 0.001$.

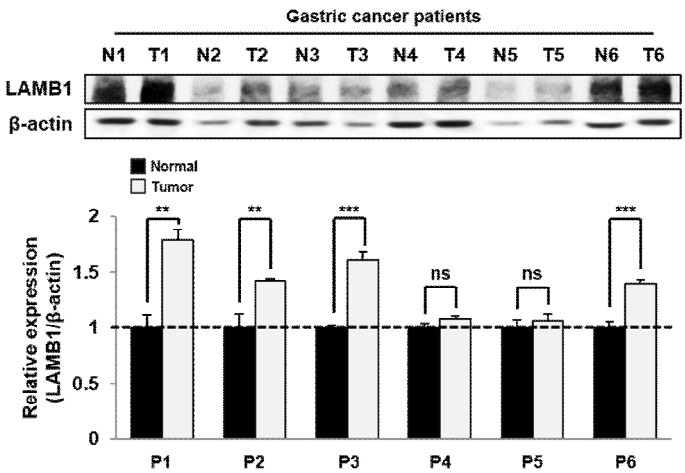

Figure 6. LAMB1 is upregulated in gastric cancer patient tissues. The protein expression of LAMB1 in six pair patients of gastric normal tissues and tumor tissues were performed by western blotting. Relative protein expression was measured and calculated by using the program of Image J. β-actin was used as the control. Presenting of all data as mean ± SEM. $^{**}P < 0.01$; $^{***}P < 0.001$; ns: not significant.

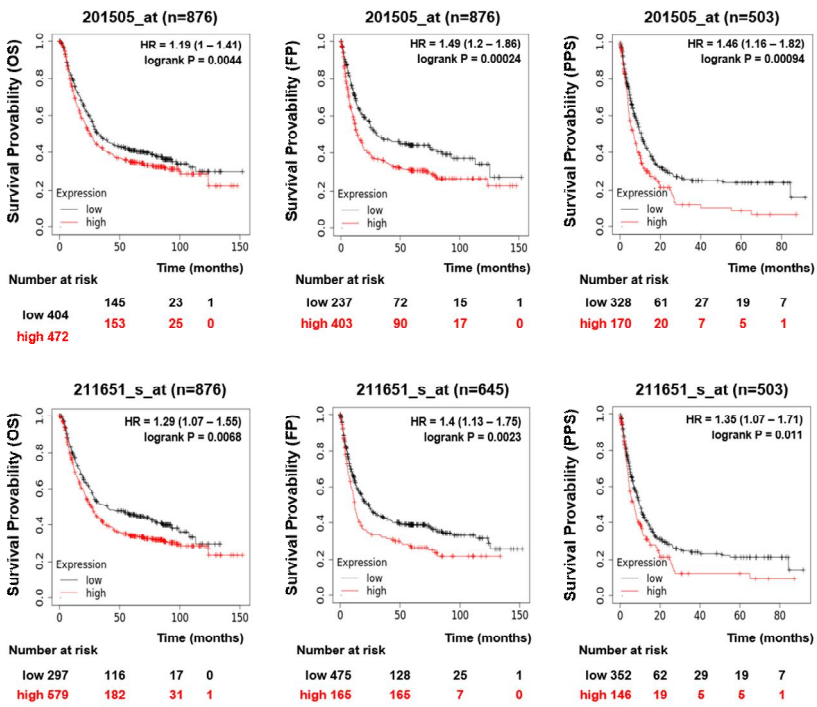

Figure 7. LAMB1 is related to poor prognosis in gastric cancer patients. Survival curves were produced by public database (201505_at and 211651_s_at) of gastric cancer patients by KM-plot. The overall survival (OS), first progression (FP) and post progression survival (PPS) for LAMB1 expression is related to poor survival.

III-3. Silence of LAMB1 inhibits the proliferation, invasion and migration in gastric cancer cells

The mRNA and protein expression of LAMB1 were confirmed by RT-PCR and western blotting in normal gastric cancer cell (GSE-1) and six gastric cancer cell lines (AGS, MKN-28, YCC-2, SNU-216, SNU-601, and SNU-668). The results showed that expression of LAMB1 was upregulated in gastric cancer cell and was similar pattern of gene and protein levels (Figure 8). To identify the biological role of LAMB1 in gastric cancer, AGS and MNN-28 cells were used for knockdown of LAMB1 using designed siRNAs (Figure 9). To investigated that the effect of knockdown of LAMB1 regulated cell proliferation in gastric cancer cells, the cells were transfected with scRNA or LAMB1 siRNA#1, #2 for 48 h. The knockdown of LAMB1 decreased cell proliferation in AGS and MNN-28 cells by colony formation assay (Figure 10). The cell proliferation is correlated with regulation of cell cycle, so cell cycle deregulation promotes the aberrant cell proliferation that characterizes cancer cell [38]. But, the cell cycle analysis showed that the silence of LAMB1 regulated the proliferation through independent cell cycle arrest (Figure 11). To determine whether LAMB1 expression were influences cell motility in gastric cancer, the transwell assay were performed for ability of invasion and migration. The results showed that silence of LAMB1 decreased cell invasion and migration in AGS and MKN-28 cells (Figure 12 and 13). The results indicated that silence of LAMB1 suppressed biological role of proliferation, invasion and migration in gastric cancer cells.

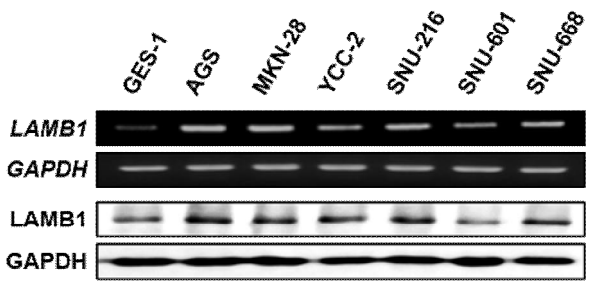

Figure 8. LAMB1 is upregulated in gastric cell lines. The mRNA and protein expression was confirmed by using RT-PCR and western blotting in gastric epithelial cell (GSE-1) and six gastric cancer cell lines (AGS, MKN-28, YCC-2, SNU-216, SNU-601 and SNU-668). The expression of LAMB1 was overexpressed in gastric cancer cells. GAPDH was used as the control.

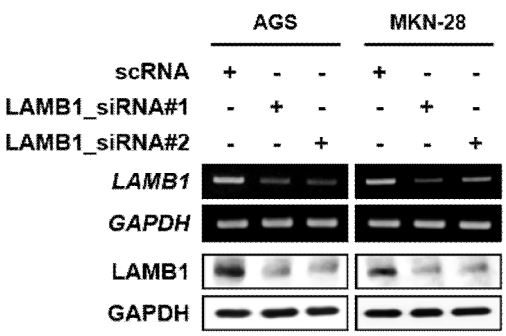

Figure 9. Expression of LAMB1 is silenced by using siRNAs. The mRNA and protein expression were performed in AGS and MKN-28 cells with scrambled siRNA (scRNA) or LAMB1 siRNA#1 and #2 transfection by RT-PCR and western blotting. Expression of LAMB1 was regulated by using LAMB1 siRNA#1 and #2. GAPDH was used as the control.

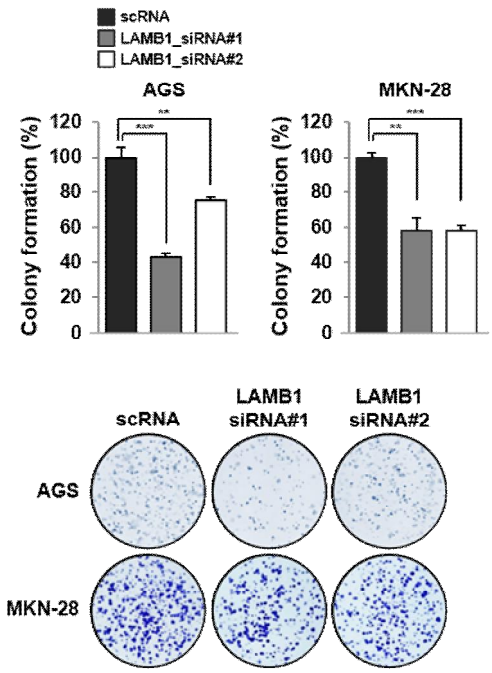

Figure 10. Silence of LAMB1 decreases proliferation in gastric cancer cells. Colony formation assay were performed for showing the cell proliferation in gastric cancer cells. The cells were incubated for 10 days and the formed colonies were stained by crystal violet solution. LAMB1 silencing inhibited the proliferation in AGS and MKN-28 cells. Presenting of all data as mean ± SEM (n = 3). $^{**}P < 0.01$; $^{***}P < 0.001$.

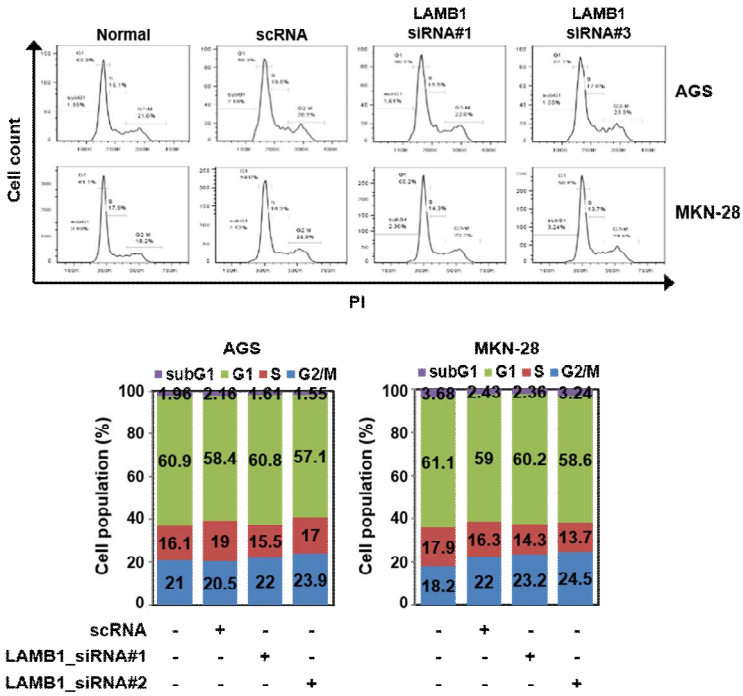

Figure 11. Silence of LAMB1 is associated with independent cell cycle arrest in gastric cancer. Cell cycle analysis were performed in AGS and MKN-28 cells. The cells were trnsfected using LAMB1 siRNA#1 and #2. LAMB1 silencing regulated cell growth through independent cell cycle arrest. The percentage of cell population was showed to bottom panel.

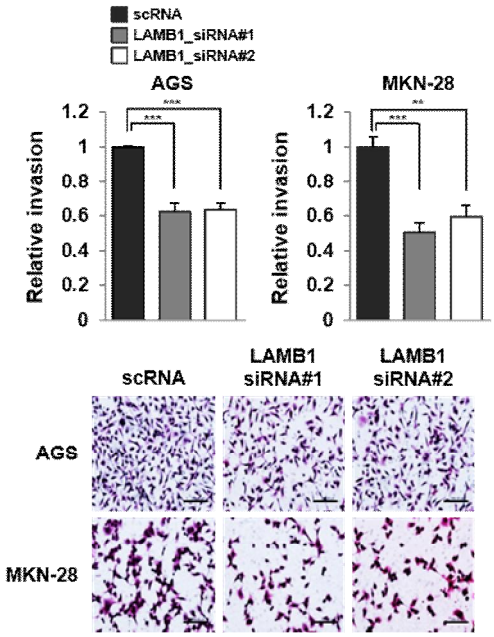

Figure 12. Silence of LAMB1 suppresses invasion of gastric cancer cells. Invasion assay was performed by using AGS and MKN-28 cells. The cells were transfected with scrambled siRNA (scRNA) or LAMB1 siRNA#1 and #2. The invading cells were stained by H&E staining. Silence of LAMB1 decreased the invasion in AGS and MKN-28 cells. Image magnification × 200; scale bar, 50 μm. Presenting of all data as mean ± SEM (n = 3). $^{**}P < 0.01$; $^{***}P < 0.001$.

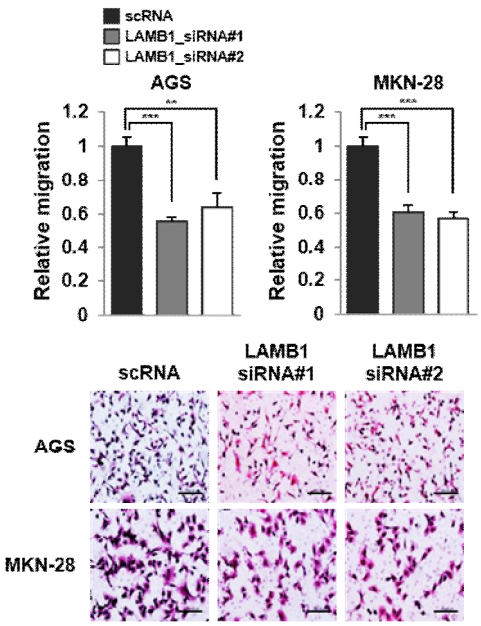

Figure 13. Silence of LAMB1 inhibits migration of gastric cancer cells. Migration assay was performed by using AGS and MKN-28 cells. The cells were transfected with scrambled siRNA (scRNA) or LAMB1 siRNA#1 and #2. The migrating cells were stained by H&E staining. Silence of LAMB1 decreased the migration in AGS and MKN-28 cells. Image magnification × 200; scale bar, 50 μm. Presenting of all data as mean ± SEM (n = 3). $^{**}P < 0.01$; $^{***}P < 0.001$.

III-4. Overexpression of LAMB1 promotes the proliferation, invasion and migration in gastric cancer cells

To investigate the effect of LAMB1 overexpressed in gastric cancer cells, SNU-601 and SNU-668 cells were transfected with mock vector or LAMB1 overexpression vector for 48 h. I showed the efficiency of LAMB1 overexpression vector in SNU-601 and SNU-668 cells (Figure 14). Colony formation assay showed that the overexpression of LAMB1 increased the number of colonies. Thus, high expression of LAMB1 promoted the proliferation in transfected SNU-601 and SNU-668 cells (Figure 15). Also, overexpressed LAMB1 significantly enhanced cell motility of invasion and migration in SNU-601 and SNU-668 cells by using transwell assay (Figure 16 and 17). Above of data suggested that overexpression of LAMB1 induced cell proliferation, invasion and migration in gastric cancer cells.

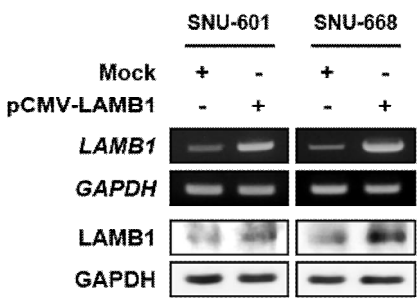

Figure 14. LAMB1 expression is overexpressed by using overexpression vector. The mRNA and protein expression were performed in SNU-601 and SNU-668 cells with pCMV-3Tag-1A vector (Mock, empty vector) or pCMV-3Tag-1A-LAMB1 (pCMV-LAMB1, LAMB1 overexpression vector) by RT-PCR and western blotting. LAMB1 was overexpresssed by using LAMB1 overexpression vector. GAPDH was used as the control.

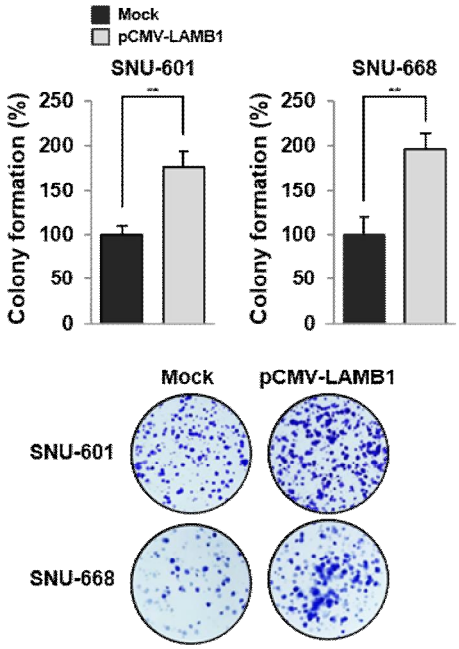

Figure 15. Overexpression of LAMB1 promotes proliferation of gastric cancer. Colony formation assay were performed in SNU-601 and SNU-668 cells. The cells were transfected and incubated for 10 days. The formed colonies were stained by crystal violet solution. Overexpression of LAMB1 promoted the proliferation in SNU-601 and SNU-668 cells. Presenting of all data as mean ± SEM (n = 3). $^{**}P < 0.01$.

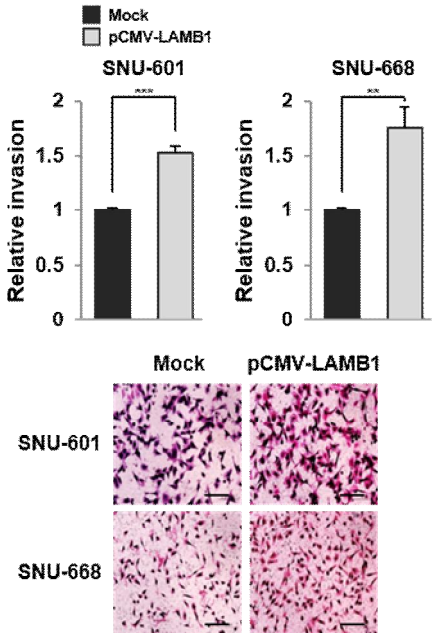

Figure 16. Overexpression of LAMB1 promotes invasion of gastric cancer cells. Invasion assay was performed by using SNU-601 and SNU-668 cells. The cells were transfected with pCMV-3Tag-1A vector (Mock, empty vector) or pCMV-3Tag-1A-LAMB1 (pCMV-LAMB1, LAMB1 overexpression vector). The invading cells were stained by H&E staining. Overexpression of LAMB1 increased the invasion in SNU-601 and SNU-668 cells. Image magnification × 200; scale bar, 50 μm. Presenting of all data as mean ± SEM (n = 3). $^{**}P < 0.01$; $^{***}P < 0.001$.

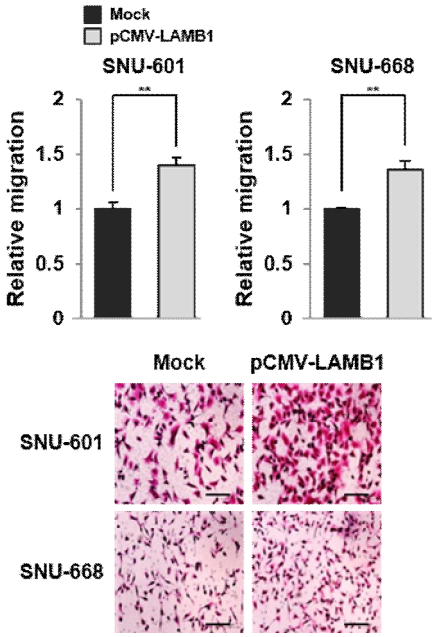

Figure 17. Overexpression of LAMB1 increases migration of gastric cancer cells. Migration assay was performed by using SNU-601 and SNU-668 cells. The cells were transfected with pCMV-3Tag-1A vector (Mock, empty vector) or pCMV-3Tag-1A-LAMB1 (pCMV-LAMB1, LAMB1 overexpression vector). The migrating cells were stained by H&E staining. Overexpression of LAMB1 increased the migration in SNU-601 and SNU-668 cells. Image magnification × 200; scale bar, 50 μm. Presenting of all data as mean ± SEM (n = 3). $^{**}P < 0.01$.

III-5. U0126 decreases cell growth and motility by regulating the LAMB1 expression

In hepatocellular carcinoma (HCC), it is reported that LAMB1 is regulated through ERK and Akt pathway [25]. ERK pathway majorly regulate the LAMB1 expression in HCC. To identify the effect of ERK pathway on LAMB1 expression in gastric cancer, AGS and MKN-28 cells were used and the cells were treated with U0126 of ERK inhibitor. The protein expression of LAMB1 was decreased in U0126 treatment compared with DMSO treatment of cells (Figure 18). To identify whether U0126 regulate cell growth and motility in gastric cancer, U0126 inhibited the biological function of cell proliferation, invasion and migration in gastric cancer cells (Figure 19, 20 and 21). To examined whether the recovery of LAMB1 expression had an effect on increasing cancer ability of proliferation, invasion and migration, the cells were transfected with LAMB1 overexpression vector in U0126 pre-treated AGS and MKN-28. The protein expression of LAMB1 was recovered by overexpression vector (Figure 18). The recovery of LAMB1 expression significantly elevated the cell proliferation in U0126 pre-treated AGS and MKN-28 cells (Figure 19). Also, the cell invasion and migration was increased (Figure 20 and 21). The results indicated that LAMB1 expression was regulated by ERK pathway, and affected cancer biological function of cell proliferation, invasion and migration in gastric cancer.

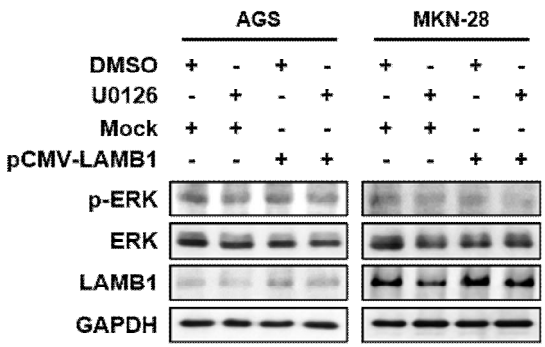

Figure 18. U0126 regulates expression of LAMB1 in gastric cancer cells. The protein expression were performed in AGS and MKN-28 cells. U0126 was treated in AGS and MKN-28 cells. And the cells were transfected with pCMV-3Tag-1A vector (Mock, empty vector) or pCMV-3Tag-1A-LAMB1 (pCMV-LAMB1, LAMB1 overexpression vector). The expression of P-ERK, ERK and LAMB1 were detected by western blotting. LAMB1 was regulated by U0126. GAPDH was used as the control.

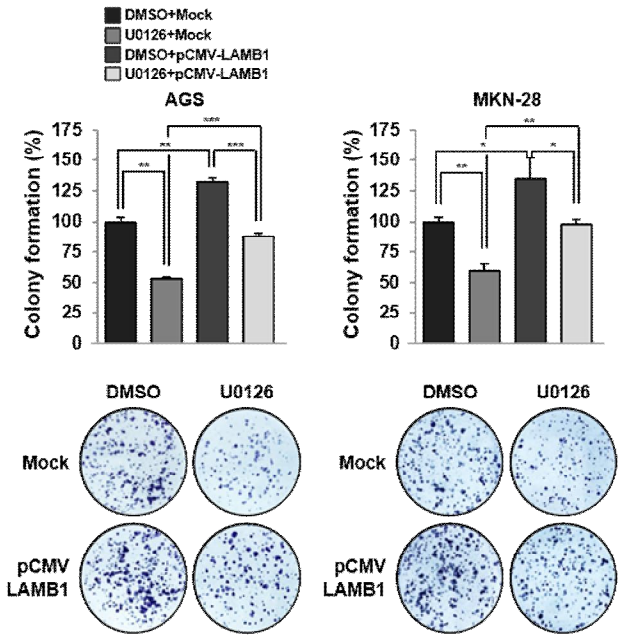

Figure 19. U0126 decreases proliferation by regulating the LAMB1 expression in gastric cancer cells. Colony formation assay were performed in AGS and MKN-28 cells. The cells were treated to U0126, and then the cells were transfected with LAMB1 overexpression vector and incubated for 10 days. The formed colonies were stained by crystal violet solution. Presenting of all data as mean ± SEM (n = 3). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

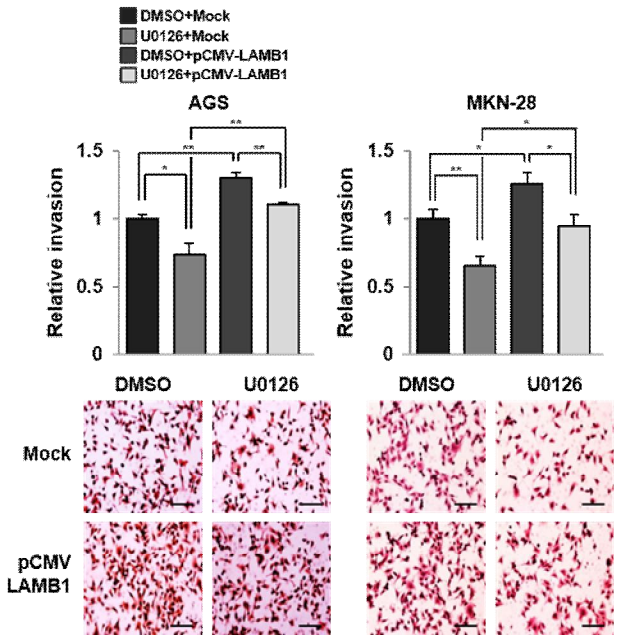

Figure 20. U0126 decreases invasion by regulating the LAMB1 expression in gastric cancer cells. Invasion assay was performed by using AGS and MKN-28 cells. The cells were treated to U0126, and then the cells were transfected with pCMV-3Tag-1A vector or LAMB1 overexpression vector. The invading cells were stained by H&E staining. Image magnification × 200; scale bar, 50 μm. Presenting of all data as mean ± SEM (n = 3). $^{*}P < 0.05$; $^{**}P < 0.01$.

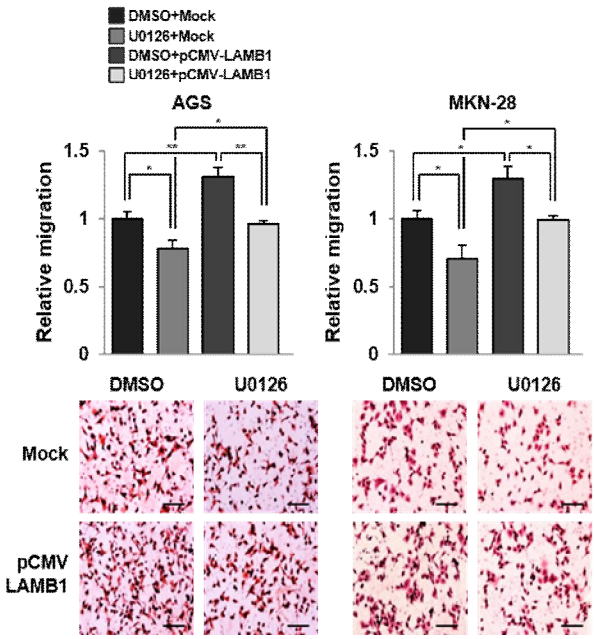

Figure 21. U0126 decreases migration by regulating the LAMB1 expression in gastric cancer cells. Migration assay was performed by using AGS and MKN-28 cells. The cells were treated to U0126, and then the cells were transfected with pCMV-3Tag-1A vector or LAMB1 overexpression vector. The migrating cells were stained by H&E staining. Image magnification × 200; scale bar, 50 μm. Presenting of all data as mean ± SEM (n = 3). $^{*}P < 0.05$; $^{**}P < 0.01$.

III-6. c-Jun binds to LAMB1 promoter and mediates LAMB1 transcription activity

The transcription factor that regulate LAMB1 gene expression remains unknown. PROMO 3.0. was used to identify the transcription factors to LAMB1 promoter. The prediction program showed the potential various transcription factors in LAMB1 promoter, including c-Jun, C/EBP β, RXR α, XBP-1 and YY1 (Figure 22). To investigate the effect of potential transcription factor in the regulation of LAMB1 expression in gastric cancer cells, i performed the knockdown using siRNAs. The efficiency of various siRNAs was confirmed by RT-PCR (silence of c-Jun using designed siRNA #1) (Figure 23.A). The results showed that silence of c-Jun and XBP-1 effectively inhibited the mRNA expression of LAMB1 in AGS cells (Figure 23.B). It is reported that the expression of ERK/ c-Jun axis in cancer cells mediates the cellular process of cell growth, motility and apoptosis [39]. XBP-1 also promotes the tumor growth, but, is regulated through PI3K/Akt pathway [40]. And public GEO dataset of GSE63089 showed that LAMB1 expression was correlated with c-Jun expression (Figure 24). In addition, U0126 of ERK inhibitor decreased the protein expression of c-Jun (Figure 25.A). To investigate whether LAMB1 expression was regulated by ERK/c-Jun axis, AGS and MKN-28 cells were pretreatment of U0126 for 24 h and transfected with mock vector or pCMV-LAMB1 overexpression vector. From the above results, c-Jun was regulated through ERK pathway and ERK pathway and c-Jun were upstream of LAMB1 in gastric cancer cells (Figure 25.B). The c-Jun binding motif was showed several promoter binding sites in LAMB1 promoter (< 2000bp) region. I selected sites where the c-Jun could bind potential sites and design two binding sites (-1403 to -1225bp and -1046 to -851bp). Chip assays showed that the c-Jun directly bound to the LAMB1 promoter in gastric cancer (Figure 26). And, silence of c-Jun decreased gene and protein expression of LAMB1 in gastric cancer cells (Figure 27). These results concluded that c-Jun directly binds to LAMB1 promoter and regulated gene expression of LAMB1 through ERK pathway in gastric cancer cells.

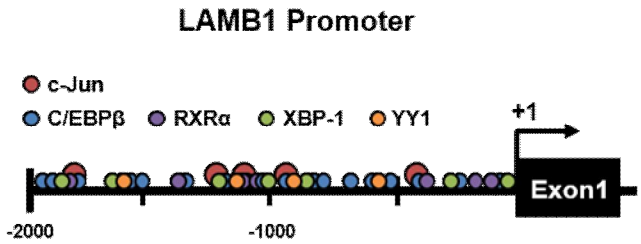

Figure 22. The potential transcription factors binds to the LAMB1 promoter in gastric cancer cells. Schematic representation showed the potential transcription factor in LAMB1 promoter (< 2000bp) region by using PROMO 3.0. The potential transcription factor were c-Jun, C/EBP β, RXR α, XBP-1 and YY1.

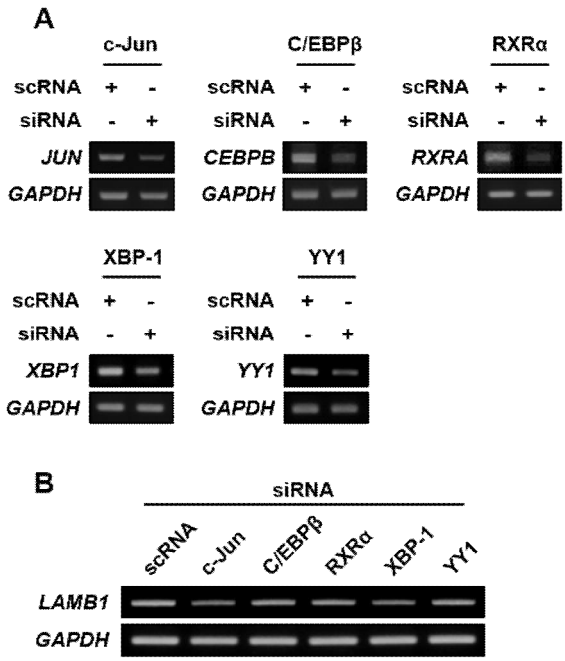

Figure 23. c-Jun and XBP-1 inhibit gene expression of LAMB1. AGS cells were transfected with scrambled siRNA (scRNA) or various siRNA (used siRNA of c-Jun, C/EBP β, RXR α, XBP-1 and YY1) for 48 h. (**A**) The efficiency of various siRNAs was confirmed by RT-PCR. (**B**) c-Jun and XBP-1 regulated the gene expression of LAMB1 in AGS cells. GAPDH was used as the control.

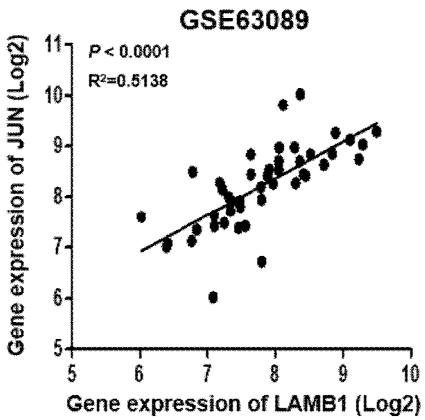

Figure 24. JUN and LAMB1 are associated with correlation between gene expression in public GSE data. Public GSE data of GSE63089 was anlyzed by using GraphPad Prism5 software. The gene expression of c-Jun was associated with gene expression of LAMB1 in gastric cancer.

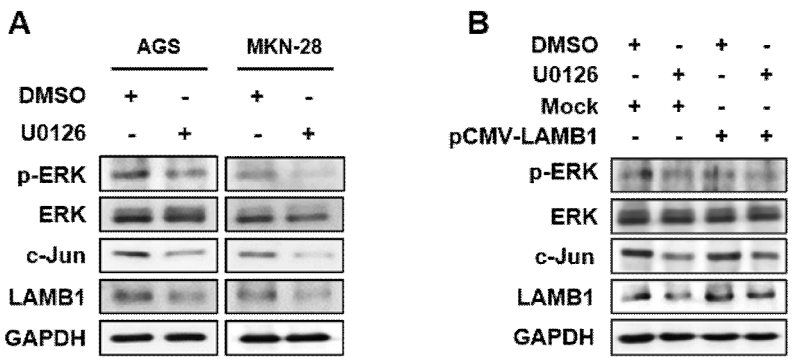

Figure 25. U0126 regulates expression of c-Jun in gastric cancer cells. The protein expression were performed in AGS and MKN-28 cells. U0126 were treated in AGS and MKN-28 cells. (**A**) U0126 regulated the c-Jun expression in gastric cancer cells. (**B**) Treated cells were transfected with pCMV-3Tag-1A vector (Mock, empty vector) or pCMV-3Tag-1A-LAMB1 (pCMV-LAMB1, LAMB1 overexpression vector). The expression of P-ERK, ERK, c-Jun and LAMB1 were detected. GAPDH was used as the control.

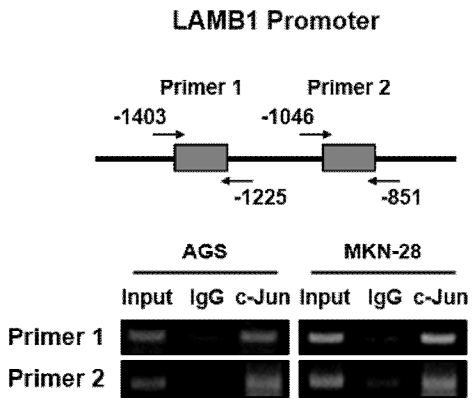

Figure 26. c-Jun directly binds to the LAMB1 promoter as a transcription factor. Chromatin immunoprecipitation (ChIP) assay showed that c-Jun directly bound to LAMB1 promoter (designed primer 1, -1403 to -1225bp and primer 2, -1046 to -851bp). lgG (negative control) and input (positive control) were used as the control.

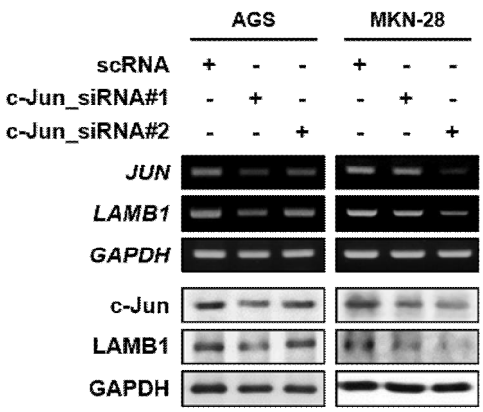

Figure 27. Silence of c-Jun inhibits the mRNA and protein expression of LAMB1 in gastric cancer cells. The mRNA and protein expression were performed in AGS and MKN-28 cells with scrambled siRNA (scRNA) or c-Jun siRNA#1 and #2 transfection by RT-PCR and western blotting. c-Jun regulated the mRNA and protein expression of LAMB1 in gastric cancer cells. GAPDH was used as the control.

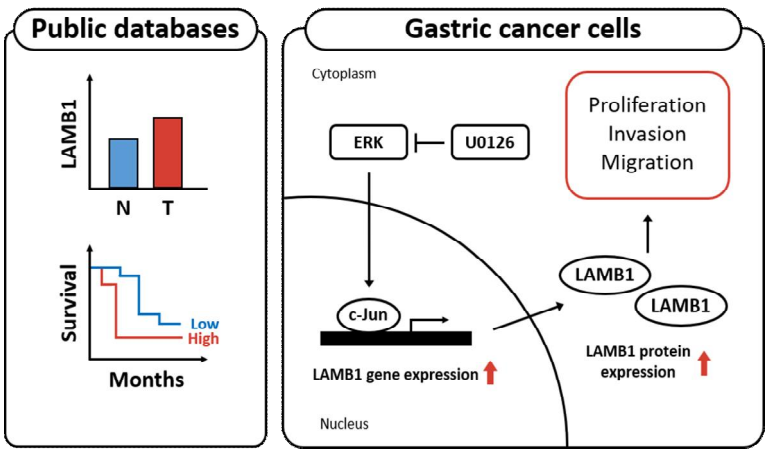

Figure 28. Overview of LAMB1 expression in gastric cancer. The expression of LAMB1 was upregulated in gastric cancer and associated with poor prognosis in gastric cancer patients. ERK pathway regulated LAMB1 expression and c-Jun directly binds the LAMB1 promoter in gastric cancer cells. Thus, The ERK/c-Jun pathway promoted the expression of LAMB1 in gastric cancer cells. The overexpression of LAMB1 promoted biological function of cell proliferation, invasion and migration in gastric cancer cells.

IV. DISCUSSION

Gastric cancer is important the global burden of cancer [6]. Because of the methods of diagnostic and surgical techniques, recently the survival rate is improved in gastric cancer patients. Until recently, it is important to explore the efficiently diagnostic and prognostic biomarker of gastric cancer. The metastasis, is the hallmark of cancer and highly cause of deaths in cancer patients, is one of the most therapeutic target in various cancer, including gastric cancer [41, 42]. Metastasis has a multi process that invasion and migration and this process involved the mechanism of cytoskeleton and adhension in cancer [43]. In addition, the metastatic cells interact with other cells or proteins and promoted the tumor microenvironment [42]. It is reported that the progression of epithelial-mesenchymal transition (EMT) is required for metastasis initiation and tumor formation [44]. The extracellular matrix (ECM) components, including laminin, are important regulator of EMT process and tumor invasion and metastasis [45]. It is reported that the gene expression of LAMB1 is upregulated in several invasive cancers [14]. In public GEO datasets of gastric cancer, the overexpressed genes, including LAMB1, are related to pathway of cell adhension and ECM interaction. Thus, the potential biomarker and cellular mechanism of LAMB1 is explored in gastric cancer cells.

First, take study showed that gene and protein expression of LAMB1 is upregulated in publicial datasets of gastric cancer and gastric cancer patient tissues. And overexpression of LAMB1 caused poor prognosis of survival rate in gastric cancer patients. Laminin subunit family promote tumor growth and progression in cancer [21-24]. The LAMB1 also has a function of tumor progression at the invasion in HCC [46]. In our results, silence of LAMB1 inhibited the growth and motility in gastric cancer cells, whereas overexpression of LAMB1 significantly enhanced the proliferation, invasion and migration in gastric cancer cells. The LAMB1 has biological function of tumor growth and motility in gastric cancer cells.

The MAPK/ERK signaling pathway has important roles in gastric cancer, such as tumor genesis and progression [47]. Also, ERK pathway lead to active the invasion, migration and

metastasis progression of malignant cancer. The cell-laminin interaction is important process in signal transduction pathways and is related in kinase-phosphatase cascades stimuli gene expression and cellular function [48, 49]. Recently, it is reported that the interaction of integrin and ECM promote intracellular pathway of phosphorylated FAK and ERK signaling in AGS and MKN-74 of human gastric cancer cells [50]. The expression of LAMB1 is regulated by PDGF signaling activated MAPK/ERK and PI3K/Akt pathway in HCC. MAPK/ERK pathway efficiently the activation of LAMB1 express more than PI3K/Akt pathway [25]. I found that ERK pathway regulates LAMB1 expression and promote the elevation of cell growth and motility in gastric cancer cells. The inhibition of ERK pathway is also potential therapeutic target marker in gastric cancer with LAMB1 expression.

The gene and protein expression of LMAB1 is upregulated in gastric cancer tissues and cell lines. In HCC, the cytoplasmic La affect the translation of LAMB1 in cells [25]. However, it is not yet known to the transcription factor of LAMB1 in human cancer. In this study focus to reveal a transcription factor of LAMB1 in gastric cancer. It is reported that transcription factor of AP-1 complex binds and regulates expression of LAMB1 in F9 mouse embryonal cell and LAMC2 in HT29 human colon cancer cell [51, 52]. And AP-1 complexes are activated by the MAP kinase family [53]. The c-Jun of AP-1 complex promote expression of genes associated with ECM components and mediate cell growth and invasion in cancer [35, 36]. Also, c-Jun regulates expression of laminin [37]. But, the transcriptional role of c-Jun regulates LAMB1 gene expression in gastric cancer didn't have been studied. The results observed that the correlation with LAMB1 and c-Jun gene expression are highly related, and c-Jun binds the LAMB1 promoter regions and is role as a transcription factor to regulate gene expression of LAMB1 in gastric cancer, which must need to be explored the detail function in further.

In addition, in this study found that the prediction of transcription factors is not only c-Jun but also XBP-1. The silence of c-Jun and XBP-1 effectively regulated LAMB1 (Figure 23.B). XBP-1, X-box binding protein-1, has been reported function as a transcription factor under ER stress conditions by glucose uptake and hypoxia [54]. In addition, XBP-1 involved in activation of cell proliferation and metastasis in cancer [55-56]. And PI3K/Akt pathway regulated the XBP-1 expression [57]. In this study, XBP-1 also

regulates the LAMB1 expression like c-Jun in gastric cancer, but isn't regulated by ERK pathway. And, it remains undefined that the XBP-1 directly bind the LAMB1 promoter and regulate gene expression of LAMB1 in gastric cancer, which need to further study.

In this study, LAMB1 was upregulated in gastric cancer and associated with poor prognosis in gastric cancer patients. LAMB1 was regulated by MAPK/ERK pathway. c-Jun was downstream of MAPK/ERK pathway and has a role of transcription factor that binds the LAMB1 promoter, promoting the gene and protein expression of LAMB1. And high expression of LAMB1 increased the biological function of proliferation, invasion and migration in gastric cancer cells (Figure 28). It is reported that elevated LAMB1 is resulted in increased secretion of laminin111 (Ln-111) in supernatant [42]. So, the overexpression of LAMB1 may increase laminin expression in supernatant, promoting tumor progression and metastasis, and serve as important marker in gastric cancer. In conclusion, LAMB1 was significantly overexpressed in gastric cancer and promoted tumor growth and motility of invasion and migration in gastric cancer cells. Indeed, the results propose that LAMB1 is a potential therapeutic target of gastric caner growth and metastasis and is predictive biomarker for prognosis and prediction in gastric cancer.

V. ABSTRACT

The mechanism study of LAMB1 as diagnostic and prognostic biomarker for gastric cancer

Hana Lee
Advisor : Prof. Seok-Jun Kim, Ph.D.
Department of Integrative Biological Sciences
Graduate School of Chosun University

Gastric cancer is the fifth most common cancer worldwide. The survival rate is poor rates of around 20%. The survival rate is poor rates of around 20% in worldwide. And the methods of diagnosis and prognosis of gastric cancer remains poor. To explore the gene that is a potential biomarker of gastric cancer, i searched and analyzed by using public GEO datasets. The analysis of GEO2R was showed that 56 genes were upregulated in used GSE data. To confirm LAMB1 protein expression in gastric cancer patient tissues, protein expression of LAMB1 also overexpressed of gastric cancer tissues. In addition, i found that high expression of LAMB1 was related with poor survival rate in gastric cancer patients. It is reported that LAMB1 has a biological function of tumorigenesis and metastasis in hepatocellular carcinoma. However, the mechanism function of LAMB1 remains undefined in gastric cancer.

To explore the biological function of LAMB1 during tumor growth and motility in gastric cancer, i used small interfering RNAs for knockdown of expression level of LAMB1. Silence of LAMB1 inhibited the cell proliferation, invasion and migration in gastric cancer cells. In contrast, overexpression of LAMB1 level promoted opposing these effects of cell growth and motility in gastric cancer. Next to confirm the signaling pathway, which regulated the LAMB1 expression in gastric cancer, i searched that the already public

study investigated that MAPK/ERK signaling could regulate the expression of LAMB1. To identify the effect of ERK pathway that regulate LAMB1 expression in gastric cancer, U0126 (ERK pathway inhibitor) was treated in gastric cancer cells. The results indicated that LAMB1 expression was regulated by ERK pathway, and affects cancer biological function of cell growth and motility in gastric cancer. In addition, to identify the transcription factors that regulate expression of LAMB1 by using prediction program. The results showed the potential various transcription factors in LAMB1 promoter. And using ChIP assay showed that the transcription factor of c-Jun could directly bind to LAMB1 promoter region and mediate gene and protein expression of LAMB1 in gastric cancer.

Take the results indicated that LAMB1 is upregulated in gastric cancer tissues compared to normal tissues, and play a risk of poor survival rate on gastric cancer patients. In addition, the LAMB1 expression increases the proliferation, invasion and migration in gastric cancer cells through ERK/c-Jun pathway. Therefore, i propose that regulation of LAMB1 is a potential therapeutic target of gastric cancer growth and motility, and is predictive biomarker for prognosis and prediction in gastric cancer.

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