Master's Thesis

Long non-coding RNA *SENCR* is a positive regulator of *ETV2*

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2017

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A thesis
submitted to the Graduate School of UNIST
in partial fulfillment of the
requirements for the degree of
Master of Science

Yujin Jeong

7/20/2017 of submission

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7/20/2017

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Abstract

Although long non-coding RNAs (lncRNAs) have emerged as novel regulator of cell fate and gene expression, the regulation of vascular specific transcription factor by lncRNA in generation of induced endothelial cells (iEndo) has not been studied yet. In this study, ETS variant 2 (*ETV2*) converts human fibroblasts into iEndo, and smooth muscle and endothelial cell enriched migration/differentiation-associated long non-coding RNA (*SENCR*) was identified as a regulator of *ETV2*. iEndo showed similar morphology, endothelial cell markers, and tubular structure formation compared to human umbilical vein endothelial cell (HUVEC). Furthermore, over-expression of *SENCR* increased *ETV2* gene and protein expression by enhancing *ETV2* promoter activity through recruitment of PSPC1. This is the first study demonstrates the role of *SENCR* contributed to *ETV2* activation in generation of iEndo.

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Abbreviations

7-Aminoactinomycin D (7-AAD)

Androgen receptor (AR)

Bicinchoninic acid assay (BCA)

BMP/OP-responsive gene (BORG)

Bone morphogenetic protein (BMP)

Central polypurine tract (cPPT)

c-Myelocytomatosis oncogene (c-Myc)

Dulbecco's modified eagle medium (DMEM)

Embryonic stem cells (ESCs)

Embryoid body (EB)

Endothelial nitric oxide synthase nitric oxide synthase 3 (eNOS /NOS3)

Enhanced chemiluminescence (ECL)

Epidermal growth factor (EGF)

ETS variant 2 (ETV2, ER71, Etsrp)

Fluorescence-activated cell sorting (FACS)

Fetal bovine serum (FBS)

Fibroblast growth factor 2 (FGF2)

Fluorescein isothiocyanate (FITC)

Forkhead box C2 (FOXC2)

Forkhead box O1 (FOXO1)

Friend leukemia integration 1 (FLI1)

GATA binding protein 2 (GATA2)

Gene ontology (GO)

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

Growth arrest specific 5 (GAS5)

Induced pluripotent stem cells (iPSCs)

Insulin-like growth factor (IGF)

Kinase insert domain receptor, fetal liver kinase-1, VEGF receptor 2 (KDR, FLK1, VEGFR2)

Kruppel-like factor 2/4 (Klf2/4)

Human embryonic kidney cells 293 that express the SV40 large T antigen (HEK293T)

Human umbilical vein endothelial cell (HUVEC)

Horseradish peroxidase (HRP),

LIM domain only 2 (LMO2)

Liquid chromatography–mass spectrometry (LC-MS)

Long non-coding RNA (lncRNA)

Long terminal repeats (LTRs)

Minimum Essential Medium (MEM)

Myocardial infarction associated transcript (MIAT)

Micro RNA (miRNA)

Mouse embryonic fibroblasts (MEFs)

Mouse tail-tip fibroblasts (TTFs)

Myogenic differentiation 1 (MyoD1)

NK2 homeobox 5 (NKX2.5)

Non-essential amino acids (NEAA)

Paraformaldehyde (PFA)

Paraspeckle component 1 (PSPC1)

Phosphate buffered saline (PBS)

Platelet derived growth factor receptor alpha (PDGFα)

Platelet endothelial cell adhesion molecule/cluster of differentiation 31 (PECAM-1/CD31)

POU class 5 homeobox 1 (Oct3/4)

Polypyrimidine tract-binding protein 1 (PTBP1)

Quantitative PCR (qPCR)

RNA polymerase II transcription (Pol II)

Room temperature (RT)

Smooth muscle and endothelial cell enriched migration/differentiation-associated long non-coding RNA (SENCR)

Small nucleolar RNA (snoRNA)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Spleen focus-forming virus promoter (SFFV)

SRY (sex determining region Y)-box2/7 (Sox2/7)

T cell acute lymphocytic leukemia 1 (TAL1)

TEK receptor tyrosine kinase (TEK, TIE2)

Transfer RNA (tRNA)

SENCR variant 1/2 (S1/2)

Vascular endothelial cadherin/Cluster of differentiation 144 (VE-cadherin/CD144)

Vascular endothelial growth factor (VEGF)

Von Willebrand factor (vWF)

Woodchuck hepatitis virus post-transcription regulatory element (WPRE)

X-inactive specific transcript (XIST)

I. Introduction

1. Pluripotent stem cells

Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass of blastocysts. The essential characteristics of ESCs are self-renewal and pluripotency[1]. In 1998, human blastocyst-derived, ESCs having normal karyotypes, high levels of telomerase activity, pluripotent stem cell surface markers were established[2]. Due to its self-renewal and pluripotency, human ESCs are expected to possess a high potential to treat incurable diseases. However, human ESCs have been limited in stem cell research and therapy by a number of issue such as immune rejection[3] and ethical issues[4-5].

Induced pluripotent stem cells (iPSCs) emerged as a propitious alternatives which can avoids ethical concerns and immune rejection. Because it was generated from adult somatic cells, researchers have been able to use iPSCs as an alternative to ESCs to avoid ethical issue. In addition, since the origin of the cells is the patient's own, the problem of immune rejection is eliminated. In 2006, Yamanaka and his college developed a method to generate iPSCs by retroviral transduction of *Oct3/4*, *Sox2*, *Klf4* and *c-Myc* into mouse embryonic fibroblasts (MEFs) and mouse tail-tip fibroblasts (TTFs)[6]. Then, human iPSCs were generated from adult human fibroblast with same approach by same group[7]. iPSCs shares similar morphology with ESCs, and have similar characteristics of ESCs including self-renewal, telomerase activity marker gene expression and pluripotency. Four major factors *Oct3/4*[8-9], *Sox2*[10], *Klf4*[11] and *c-Myc*[12] which have been named as Yamanaka factors or *OSKM* factors, have been exhibited to contribute to the maintenance of pluripotency and the rapid proliferation.

Although iPSCs were spotlighted as an alternative of ESCs to generate patient-specific stem cells to study developmental biology, drug development, and a new cell-based therapies, it has not become entrenched in therapeutic application yet[13]. One of the major concerns of iPSCs is the tumorigenicity. iPSC clones transduced by retrovirus contain numerous random integrations of transduced genes that have potential to be reactivation. Approximately 20% of germline chimeric mice from iPSCs exhibited tumor occurrence result from reactivation of the *c-Myc* transgene[14]. Thus, non-integrating induction methods using adeno-viral plasmid vector[15-16], Sendi-viral plasmid vector[17], episomal vector[18-19], mRNA[20], protein[21] and transposons[22-23] have been developed to generate iPSCs. However, their efficiency and reliability is lower than the lenti- or retro-virus reprogramming approach[24], and the non-integrating induction methods are not completely free from safety risks also[25].

Numerous methods of the differentiation from PSCs into vascular cells have been established[26-29]. Conventionally, two methods are generally used to different human PSCs to vascular cells. First one is embryoid body (EB) formation method[30-31]. However, it is often time-consuming since the peak expression of endothelial genes occurs after 10–15 days of induction[32], and is inefficient (1%–5%)[31, 33-34]. The other method is monolayer-directed differentiation[35-36]. It offers increased efficiency (5–

20%), but it depends on undefined supplements, co-culture[35, 37-38], heterogeneous cell aggregates[39], conditioned medium[36] or inconstant yields of vascular cells[40].

2. Direct conversion

Direct reprogramming or direct conversion is a method to convert one cell into another with bypassing the pluripotent state. Since this method bypasses pluripotent stage, it does not require further differentiation into specific cell lineage, and also can eliminate tumorigenic potential caused by any residual undifferentiated cells[41].

The first discovery of direct conversion is the change of mouse fibroblasts into myoblasts through transfection of DNA isolated from induced or primary myoblasts[42]. Subsequent studies identified that *MyoD1*, a critical transcription factor for specification of the skeletal muscle lineage during early development, could induce muscle-specific characteristics when delivered into fibroblasts. After that, a number of studies reported conversion of one type of cells into another by delivering transcription factors, proteins of RNA, and this method is called as direct conversion or direct reprogramming[43].

Direct conversion of somatic cells such as fibroblasts into other cell types holds a great therapeutic promise. To date, many types of somatic cells such as hepatocyte[44-46], cardiomyocyte[47-48], astrocyte[49], renal tubular epithelial cell[50] and macrophage[51] as well as adult stem cells such as oligodendrocyte progenitor cell[52], hepatic stem cell[53], neural stem cell[54], epiblast stem cell[55] and hematopoietic stem cell[56] were generated from fibroblast by direct conversion.

Another notable concept of reprogramming is *in vivo* reprogramming. A number of previous studies demonstrated reprogramming of cell *in vivo* such as pancreatic acinar cells into β-cells[57], cardiac fibroblasts into cardiomyocytes[58] and astrocytes into neuroblasts[59]. Recently, *in vivo* hepatic reprogramming of myofibroblasts with adenoviral vector[60] and adeno-associated viral vector[61] were reported.

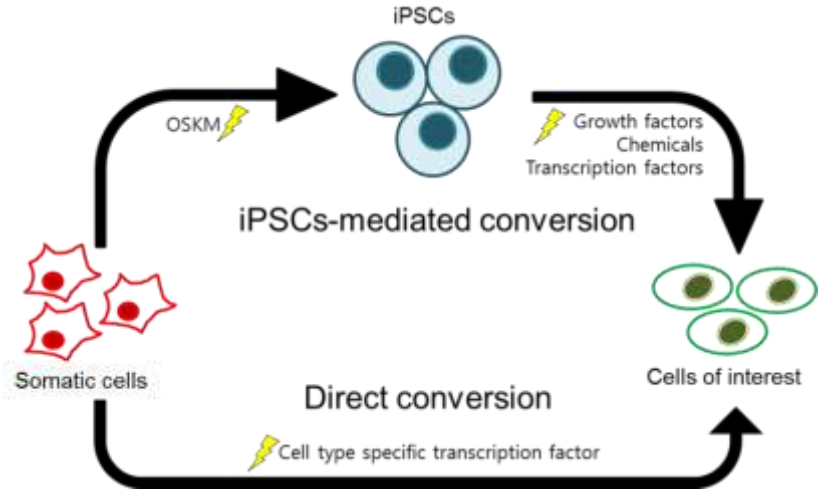


Diagram 1. Definition of direct conversion.

3. Development of blood vascular and endothelial cell

In mammalian development, vascular progenitors emerge from the lateral and posterior mesoderm[62]. The development of blood vessel system occurs by two distinct processes. One is the vasculogenesis, defined as *de novo* vessel formation through differentiation of angioblasts. The other is the angiogenesis defined as primary vessel remodeling secondary from pre-existing vessels and the stage of specification of the arterial, venous vessels[63-65].

The basic constituents of the vascular system, endothelial and blood cells are determined very early during ontogeny[64]. The first structure of endothelial and hematopoietic cells is the blood islands of the extraembryonic yolk sac at embryonic day (E) 7.5 in mice[66]. Blood islands are mesoderm cell aggregates of the common progenitor of endothelial and blood progenitor cells which is called as hemangioblast[67-68]. The inner cells become blood progenitor cells, while the outer cells become angioblast, the progenitor of blood vessel. Angioblast is a multipotent cell which is capable of giving rise to the key elements cells (pericytes, smooth muscle cells, fibroblasts) during 'angiogenesis'. Although, the concept and term are imprecise yet, this cell constitutes endothelial progenitor cell (EPC) or vascular progenitor cells (VPC). Nonetheless, researchers focused on generation of EPC or VPC *in vitro* through differentiation of PSCs and direct conversion has been progressed since these cells are proliferative and can give rise to many offspring[69-72].

The hemangioblast co-expresses *BRACHUURY (T)* and the receptor tyrosine kinase *FLK1* (*VEGFR-2, KDR*)[73]. *FLK1* is essential for yolk-sac blood-island formation and vasculogenesis in embryo[74]. BMP, Notch, and Wnt signaling is necessary for efficient *FLK1*+ mesoderm formation from ESC[75]. *ETV2* is required for the differentiation of *FLK1*/*PDGFRα* cells into vascular/hematopoietic mesoderm[76].

The synthesis of new vessels through endothelial cells has an enormous potential in clinical application such as treatment of ischemic vascular disease. However, adult stem cell therapy has problems of immune rejection and is limited in supply of donor cells[77-78]. Thus, patient-specific endothelial cell or early stage progenitor cell from induced from hPSCs or direct conversion is a promising cell source for cell therapy. Recently, two studies showed the feasibility of *OSKM* in direct conversion of fibroblast into induced endothelial cell (iEndo) (Table1).

Group	Starting cell	Final cell	TFs
Margariti, A (2012, *PNAS*)[79]	Human fibroblasts (CRL-2097, CCL-186 , CCL-153)	Partial iPSC and iEndo	*OCT4, SOX2, c-MYC, KLF4*
Li, J (2013, *Arterioscler Thromb Vasc Biol*) [80]	Human fibroblasts (CRL-2097, BJ)	iEndo	*OCT4, KLF4*

Table 1. Direct conversion from fibroblasts into iEndo using Yamanaka factors.

4. ETS variant 2 (ETV2) gene

ETS variant 2 (*ETV2*) gene, a member of erythroblast transformation-specific (ETS) family, is an important transcription factor in development of hematopoietic and endothelial lineage. Members of ETS family transcription factors have been implicated in vasculogenesis, angiogenesis, and hematopoiesis[81]. Knock-down of *Etsrp*, the zebrafish homologue to mammalian *ETV2*, resulted in major vascular defects in zebrafish[82-83]. Similarly, *Etv2* deficient mice lead to complete loss of embryonic blood and vascular structure[75] and embryonic lethality at approximately E9.0 to E9.5[75, 84].

ETV2 cooperatively interact with many transcript factors in development of hemato-endothelial lineage[85]. *GATA2* is co-expressed and interacts with *ETV2* via protein–protein interaction between *ETV2* and *GATA2* which is mediated by the interaction of Ets and Gata domains[86]. Ets factors function combinatorially with *FOXC* transcription factors through a composite DNA binding site, the FOX:ETS motif, which is bound robustly by *FOXC2* and *ETV2*, and the two proteins bind the element simultaneously[87]. *ETV2* is also regulated by other transcription factors. *ETV2* is considered as a downstream target gene of *NKX2.5*[84] and *VEGF/Flk1* signaling[88]. *Nkx2.5* binds to an evolutionarily conserved *Nkx2.5* response element in the *Etsrp71* promoter and induces the *Etsrp71* gene expression in mouse embryo. *FLK1* is upstream of *ETV2* and is necessary for *ETV2* expression. The *ETV2* promoter activity is increased by VEGF in the presence of their ligand *FLK1*. There are many other direct downstream targets of *ETV2* such as *TIE2*[84], *SOX7*[89], *LMO2*[90], *FLI1*[91] and *miR-130a*[92] that contribute endothelial lineage specification.

ETV2 has an enormous power to induce vascular endothelial cells. There are many studies that demonstrated that generation of iEndo using *ETV2* alone or *ETV2* with other transcription factors (Table 2). Also, the efficiency of endothelial cell derived from human ESCs were increased by addition of exogenous *ETV2*[93]. Furthermore, reactivation of *ETV2* transduced by lenti-virus contributed vascular repair and regeneration in adult hindlimb ischemia mouse model[94]. Similarly, reactivation of *ETV2* gene in mature iEndo induced immature iEndo[95].

Group	Starting cell	Final cell	TFs
Ginsberg, M (2012, *Cell*) [96]	Human mid-gestation amniotic cells	Endothelial cells	*ETV2*, *FLI1*, *ERG*
Han, J.K (2013, *Circulation*)[97]	Mouse adult fibroblasts	Endothelial cells	*Foxo1*, *Er71*, *Klf2*, *Tal1*, and *Lmo2*
Morita, R (2015, *PNAS*)[98]	Human adult skin fibroblasts	Endothelial cells	*ETV2*
Lee, S (2017, *Circ Res*)[95]	Human postnatal dermal fibroblasts	Early and late endothelial cells	*ETV2*

Table 2. Direct conversion of iEndo using endothelial lineage transcription factors.

5. Long non-coding RNA

Although ~90% of the eukaryotic genome is transcribed, mRNAs account only for 1%–2% of total RNAs. The rapid development and application of high-throughput deep sequencing suggested that a large number of RNA molecules are non-coding RNAs[99]. Also, the genome-wide transcriptome analyses have identified that human genome contains thousands of long noncoding RNAs (lncRNAs) [100]. These RNAs are classified as long RNA (processed transcript length >200nucleotide) that are different from short RNAs (processed transcript length <200nucleotide) such as tRNA, miRNA and snoRNA[101].

Wherase lncRNAs are types of RNA which lack protein-coding potential, transcription and post transcription modification of these lncRNAs is similar to the protein-coding mRNAs in which are transcribed by RNA polymerase II (Pol II), and transcribed RNAs are modifeied by 5′-capping, polyadenylation, and histone modifications associated with Pol II transcriptional elongation[102].

Although localization of mRNAs is specifically distributed to ribosomes in the cytoplasm, localization of lncRNA is more varied than mRNA. The location of lncRNA were identified in *XIST* on the inactive X chromosome[103], *Gomafu*, also known as *MIAT*, in the subnuclear domains[104], *BORG* restricted to the nucleus[105] and *GAS5* exported to the cytoplasm[106].

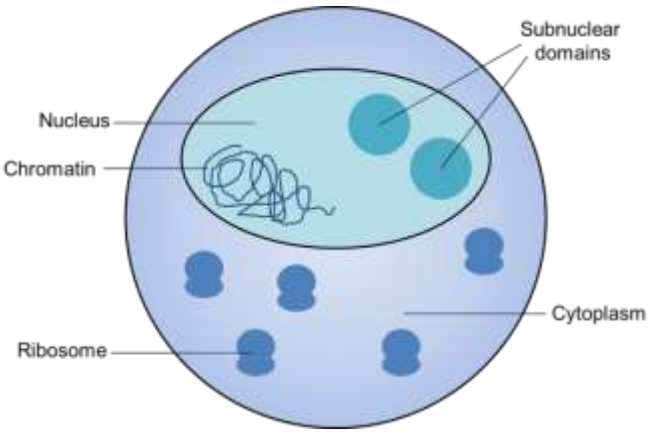


Diagram 2. Localization of lncRNA.

LncRNAs have emerged as novel regulators of gene expression and played roles in diverse biological process, such as proliferation, differentiation, and development through various modes of action[107-108]. With developmental expression patterns of tissue specific genes, lncRNA may be orchestrators and some reports have already related their role in specification of germ-layer and adult cells[109-111]. Especially, there are many studies of lncRNAs in cell-fate programming and reprogramming. Some lncRNAs have role in maintaining of the pluripotency[106, 112], adult stem cell state[113], while others promote or regulate lineage specification[112, 114]. Also, some lncRNAs regulate chromatin states for activation[115-116] or repression[117] of epigenome. Their functions are often facilitated by protein partners that impart the ability to activate or repress gene expression or posttranscriptionally regulate other RNAs.

6. Smooth muscle and endothelial cell enriched migration/differentiation-associated long non-coding RNA (SENCR)

SENCR is a human vascular-enriched lncRNA located on the chromosome 11 and exists as 2 isoforms which are a full length, *SENCR* variant 1 (S1) and an alternative spliced variant, *SENCR* variant 2 (S2)[118]. Although *SENCR* is placed in antisense orientation from within the first intron of a protein-coding gene called Friend leukemia virus integration 1 (*FLI1*), an important transcription factor of endothelial cell and blood cell formation, no significant correlation is found between *FLI1* and *SENCR* yet. Regardless of the similarity of expression of two genes in developmental time course over time, knockdown of *SENCR* with small interfering RNA (siRNA) had little or no effect on *FLI1* expression[118-119]. Also, S2 seemed to be more specific to vascularization than S1 since S1 expressed more broadly expressed in various tissues[118].

SENCR was considered as an early lncRNA in mesodermal and endothelial cell commitment and function. During endothelial cell differentiation from human ESCs, exogenously introduced *SENCR* reduced the expression of pluripotent, endodermal and ectodermal genes, but it enhanced the expression of mesodermal genes[119].

In addition to endothelial cells, *SENCR* is also involved in smooth muscle cells proliferation and migration[120]. Also, *SENCR* expression is down regulated in patients with endothelial cell dysfunction and atherosclerotic vascular disease[119]. As a result of the previous finding, *SENCR* is considered as a novel candidate target molecular for treatment of vascular diseases.

7. Objective

There are studies of lncRNAs that regulate pluripotency and differentiation. Furthermore, various lncRNAs involved in vascular function and specification have been demonstrated. LncRNA *SENCR* and *ETV2*, a transcription factor related to vasculature formation, is widely studied in several fields such as vascular specification and generation of iEndo. However, the study demonstrated the relationship of lncRNA and vascular specific transcription factor is unclear. In this study, vascular specific lncRNA *SENCR* was investigated in regulation of *ETV2*, a potent transcription factor in vascular development.

II. Materials and Method

1. Cell culture

CRL-2097 fibroblasts from the ATCC (Manassas, VA, USA) were cultured on 0.1% gelatin coated dishes (sigma, G1890) in MEM media : Minimum Essential Medium 1X (Gibco, 11095-080) supplemented with 10% fetal bovine serum (FBS) (Gibco. 10099-141), 1mM sodium pyruvate (Gibco, 11360-070), 1X MEM non-essential amino acids (NEAA, Gibco, 11140-050) and Penicillin/Streptomycin (Gibco, 15140-122). HEK293T from the ATCC (Manassas, VA, USA) (ATCC® CRL-3216™) were cultured in DMEM media: Dulbecco's Modified Eagle Medium 1X (Gibco, 10313-021) supplemented with 10% FBS, 2mM L-glutamine (Gibco, 25030-081), and Penicillin/Streptomycin. HUVEC from the ATCC (Manassas, VA, USA) (ATCC® CRL1730™) were cultured EGM2 Bullet kit (EGM2) (EBM2 supplemented with FBS, heparin, hydrocortisone, ascorbic acid, Gentamycin/amphotericin, FGF2, VEGF, IGF, and EGF) (Lonza, cc-3162).

2. Cloning plasmids

Plasmid containing human *ETV2* was a gift from RIKEN (W01A065G01). *ETV2* was amplified with Phusion High-Fidelity DNA polymerase (NEB, M05305). *SENCR* variant1 and 2 were amplified with KOD plus polymerase (TOYOBO, KOD-2011) from HUVEC cDNA. The cDNA were inserted lentiviral vector through sub-cloning using TOPO TA cloning kit with pCR2.1-TOPO plasmid (Invitrogen, 45-0641). Episomal vector plasmid (pCXLE-*hOCT3/4*-shp53-F) was a gift from Shinya Yamanaka (Addgene plasmid #27077). After remove shp53 and *hOCT3/4* region, *SENCR* variant 1 and 2 were inserted into the backbone. *ETV2* promoter sequence (+1~ –1.5kb) was amplified using KOD plus polymerase from genomic DNA of human H9 cell line. The sequence was inserted into pGL3-basic vector after TA cloning. *PSPC1* sequence was amplified from HEK293T cDNA using KOD plus polymerase. Then, it was inserted into p3XFLAG-CMV vector after TA cloning.

3. Lentivirus packaging

Before transfection, HEK293T cells were confluent with 40~60% on the 10cm plate. A lentiviral expression vector containing human ETV2 gene was co-transfected with psPAX2 and VSV-G constructs into HEK293T using X-tream Gene 9 DNA Transfection Reagent (Roche, 06365787001). Cells were incubated for 48hours 37℃. Lentivirus was harvested from the culture supernatant and concentrated using ultra-centrifuge (Beckman) for 1 hour 30minute at 80000g.

4. Endothelial-like cells induction

Human fibroblasts (HF134, CRL-2097) are seeded at 10,000 cells on 0.1% gelatin-coated 6-well dish. Next day, 4 μg/ml protamine sulfates (Sigma, P4505) were added for approximately 30min before infection. After 24 hours, media containing virus was removed and changed into EGM2 supplemented with BMP4 (Peprotech, 120-05ET), CHIR99021 (Sigma, SML1046), FGF2 (Peprotech, 100-18B), VEGF (Peprotech, 100-20) for 5 days. On Day6, media was changed into EGM2 supplemented with FGF2 and VEGF.

5. RNA extraction and cDNA synthesis

Total RNA from cultured cell lysate were extracted with RiboEx (GeneAll, 301-001). Mix vigorously with Chloroform (Merck, 102445) and centrifuge at 12000xg for 15minutes in 4℃. RNA in aqueous phase was precipitated after adding iso-propanol (Merck, 109634). After washing with 75% Ethanol (Merck, 100983) and drying, RNA was dissolved by RNase-free water. For synthesis of the complementary DNA, 1μg of RNA was transcribed with M-MuLV reverse transcriptase (NEB, M0253L) according to the manufacturer's instructions.

6. Gene expression analysis

The detection of complementary DNA expression for the specific genes was performed using reverse transcription PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR). For qRT-PCR, the relative expression of different sets of genes was quantified to GAPDH mRNA. qRT-PCR was performed using with iQ^{TM} SYBR green supermix (Bio-RAD, 170-8882AP). For, RT-PCR, taq polymerase (Thermo, 10342-020) was used according to the manufacturer's instructions.

7. Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde (PFA) (WAKO, 163-20145) in 1X PBS (Biosesang, P2007) for 10 min. Cells were washed with 0.05% Tween-20 (Sigma, P9749)/PBS (PBS-T) and permeabilized with 0.1% Triton X-100 (Sigma, T9284) in 1X PBS for 10 min. After blocking with 4% FBS/PBS for 30 min, cells were incubated with primary following antibodies; anti-PECAM (CD31) (DSHB, P2B1), anti-VE-cadherin (SantaCruz, sc-9989), anti-vWF (Abcam, AB6994) overnight at 4℃ or 1hr at RT. After washing with PBS-T and incubated with secondary antibodies conjugated with Alexa-488 or Alexa-594 (Invitrogen) and visualized by fluorescence microscopy after counterstaining with Hoechst 33342 (Thermo, 62249).

8. Tubule formation assay

Matrigel should be thawed before experiment at 4℃. 96well plates was coated with 50μl matrigel (Corning, 354234) and it should be on ice. Solidify the gel at 37℃ for 30min. $3X10^4$ cells were seeded on matigel coated 96 well plates and cultured in EGM2 supplemented with FGF2 and VEGF.

9. Western blotting

Cells were washed with PBS and lysed by RIPA buffer. Protein concentration was determined by the BCA assay (Thermo, 23228). Equal amounts of protein were separated by SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were incubated with primary antibodies, anti-ETV2 (Abcam, ab170821) and anti-β actin (Sigma, A1978), in 7% non-fat dry milk (BIO-RAD, 170-6404) overnight at 4℃. HRP- anti-rabbit and anti-mouse secondary antibodies were used as 1:5000 in 7% milk. Membranes were imaged using Clarity™ western ECL substrate (BIO-RAD, 170-5061) and a ChemiDoc™ MP imaging system (BIO-RAD).

10. Luciferase assay

Promoter activity was assessed by transiently transfecting HEK293T cells with plasmids of dual-luciferase containing firefly (pGL3-basic) and Renilla (pRL-TK) luciferase reporters using the X-tream Gene 9 transfection reagent according to manufacturer's instructions. 36 hours later, cells were lysated through passive lysis 5X buffer (Promega, E1941). After reagent with substrates was added, the luciferase activity in cell lysate was measured by GLOMAX 96 micrometer luminometer (Promega). Firefly luciferase activity was normalized to that of Renilla.

11. RNA pulldown

RNAs were synthesized from linearized pCR2.1-TOPO plasmid encoding *SENCR* variant1 and 2 through T7 RNA polymerase (Roche, 10881767001) according to the manufacturer's instructions. RNA biotinylation was carried out using biotin RNA labeling mix (10X) (Roche, 11685597910) according to the manufacturer's instructions. RNAs and protein lysate of CRL-2097 was bound at 4℃ for 2 hours. Then, washed streptavidin magnetic particles (Roche, 11641778001) was added into the mixture and bound at 4℃ for 1hours. After washing of particles which capture RNA-protein complex, 4X SDS dye and NP40 buffer (150mM NaCl, 0.5% Tween-20, 50mM Tris, pH 8.0) were added and boiled at 99℃ for 5minutes. The samples are loaded into the acrylamide gel and visualized with coomassie staining solution (0.025% Coomassie Brilliant blue R250, 40% methanol, 10% glacial acetic acid).

12. **Database searching**

Charge state deconvolution and deisotoping were not performed. All MS/MS samples were analyzed using Sequest (Thermo Fisher Scientific, San Jose, CA, USA; version 1.0) and X! Tandem (The GPM, thegpm.org; version CYCLONE (2010.12.01.1)). Sequest was set up to search the Human-2016-01_contam database (unknown version, 150364 entries) assuming the digestion enzyme stricttrypsin. X! Tandem was set up to search a reverse concatenated Human-2016-01_contam database (unknown version, 300728 entries) also assuming stricttrypsin. Sequest and X! Tandem were searched with a fragment ion mass tolerance of 1.00 Da and a parent ion tolerance of 50 PPM. C of cysteine was specified in Sequest and X! Tandem as a fixed modification.

13. **Criteria for protein identification**

Scaffold (version Scaffold_4.7.5, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 87.0% probability to achieve an FDR less than 1.0% by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 94.0% probability to achieve an FDR less than 1.0% and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii, Al et al Anal. Chem. 2003;75(17):4646-58). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Proteins were annotated with GO terms from NCBI (downloaded Feb 19, 2017) and NCBI (downloaded Feb 20, 2017). (Ashburner, M et al Nat. Genet. 2000;25(1):25-9).

Table 3. Primer sets used for gene analysis in RT-PCR and qRT-PCR.

Gene	Accession. No or Reference	Sequence
CD31	NM_000442.4	F : GCTGTTGGTGGAAGGAGTGC R : GAAGTTGGCTGGAGGTGCTC
ERG	NM_182918.3	F : TGGGCGGTGAAAGAATATGG R : TGAGGTAGTGGAGATGTGAGAG
FLK1	NM_002253.2	F : CCCCTTGAGTCCAATCACAC R : TTCCTCCAACTGCCAATACC
vWF	NM_000552.4	F : CTGGACGTGATCCTTCTCCT R : CTCAGCAAATGGGCTTTCTC
VE-CADHERIN	NM_001795.4	F : ACGGGATGACCAAGTACAGC R : ACACACTTTGGGCTGGTAGG
NOS3	NM_000603.4	F : GGGTCCTGTGTATGGATGAG R : GGGGCTGAAGATGTCTCGG
GAPDH	NM_002046.5	F : GACCCCTTCATTGACCTCAACTACATG R : GCCTTCTCCATGGTGGTGAACAC
NORAD	NR_027451.1	F : TCCTGTTTACAGCGAGGCAA R : CCATCTCCATCAACCCAGAAGA
MALAT1	NR_002819.4	F : GTGATGCGAGTTGTTCTCCG R : CTGGCTGCCTCAATGCCTAC
LINC00323	NR_024100.1	F : TTCAGGAGGAGGGTTGGTCA R : GCTCACTGCTAAGAGGAGGC
sONE	NR_133652.1	F : CTCCATGTCATCTACCTGCAC R : CTTCTGGGTGTCTGTGGTC
TUG1	NR_110492.1	F : TAACAGCCCTCCACTCCAGAT R : AGGCACCAGCTTCAAAACCC
LINC01529	NR_104176.1	F : CACTCACCTATGACCCTTAACC R : AGCTTCCTCAAACTGTTCCG
LINC01531	NR_040046.1	F : CAAGTGATTCTCCTACCTCAGC R : GCAGATCACTTTAGGTCAGGAG
TIE-AS1,3	Li *et al*, 2010, *Blood*	F : CAGCAGACACAGAAAAAGCATC R : TGGCTAGGACCTCCAGTATGAT

TIE1-AS2	Li *et al*, 2010, *Blood*	F : GATGCCCAGGATAGCTATGAG R : TGACCAGTCTGACCCTTACAG
MIR503HG	NR_024607.1	F : AGGTAGAAGGTGGGGTCTGC R : ACTGGAGGAAGCCGGATG
PUNISHER	NR_027032.1	F : GTCCTCCACTCCACCTCAAA R : TGAGTTCCTGATCGTGTCCA
LOC105375568	XR_956358.1	F : CAAAACACGTACATATGCCCTG R : GCCCTCCATACCAATAGTTCTC
LOC1079686861	XR_001758717.1	F : CAATACTGACCATCGGACTGAC R : ATTCATGTGCCTGTCCCTAAG
SENCR	NR_038908.1	F : GCTCTACCGACCTTCAAACTAC R : AGTCCTTTCTGGCTGAATGAG
ETV2	NM_014209.3	F : GGACCTGTGGAACTGGGATG R : ATGTCTCTGCTGTCGCTGTCG

III. Result

1. Direct conversion of iEndo from human fibroblasts

Several reports have demonstrated the contribution of *ETV2* in induction of endothelial cell fate. To directly convert from human fibroblasts (CRL-2097 and HF134) into iEndo, fibroblasts were transduced with lenti-viral plasmid encoding coding region of human ETV2 gene (Fig. 1.1a). Cells were infected by lenti-virus encoding human *ETV2* gene for 24 hours and cultured in endothelial induction media for 5 days. After that, the media was switched to endothelial maintenance media until endothelial morphology was emerged. Endothelial characteristics were exhibited after 14 days (Fig. 1.1b). Cells remained spindle shape at 1 day after infection. After cells were cultured in endothelial induction media for 5days, bright morphology was emerged but they still remained elongated shape. At day 14 after infection, the morphology of fibroblasts was changed into cobble-stone like shape(Fig. 1.2b). However, the control groups including no switch into endothelial media after *ETV2* infection or no *ETV2* infection with endothelial media did not change their morphology (Fig. 1.2b). iEndo expressed endothelial cell-related genes such as *CD31*, *ERG*, *FLK1*, *vWF* and *VE-CADHERIN* after day 14. However, nitric oxide synthase 3 (*NOS3*), also known as endothelial NOS (*eNOS*), was not expressed in iEndo (Fig. 1.3a). iEndo also exhibited protein expression of endothelial cell markers, vWF, VE-CADHERIN, and CD31 (Fig. 1.3b). Furthermore, iEndo formed tubular structure which is an intrinsic function of endothelial cell when cultured on matrigel-coated dishes (Fig. 1.4). These results demonstrate ectopic expression of *ETV2* alone is sufficient to directly convert fibroblasts into iEndo which exhibit primary endothelial characteristics.

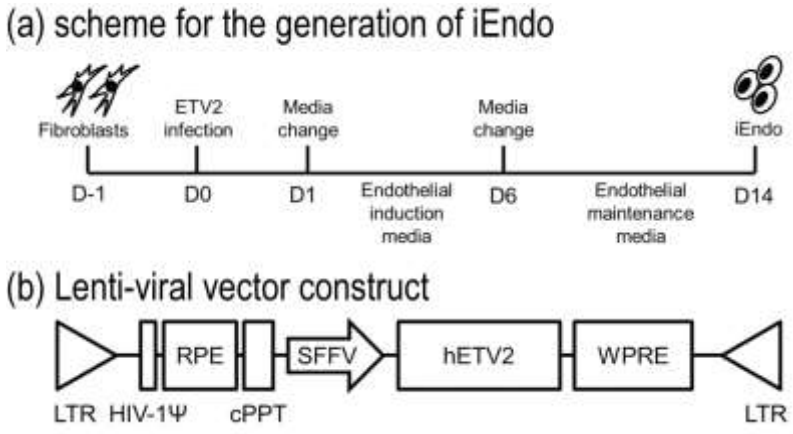


Figure 1.1 Illustrations of exprerimental procedure. (a) The scheme of direct conversion for the generation of iEndo from fibroblast by *ETV2* induction . (b) Lent-viral vector construct encoded *ETV2* transcription factor.

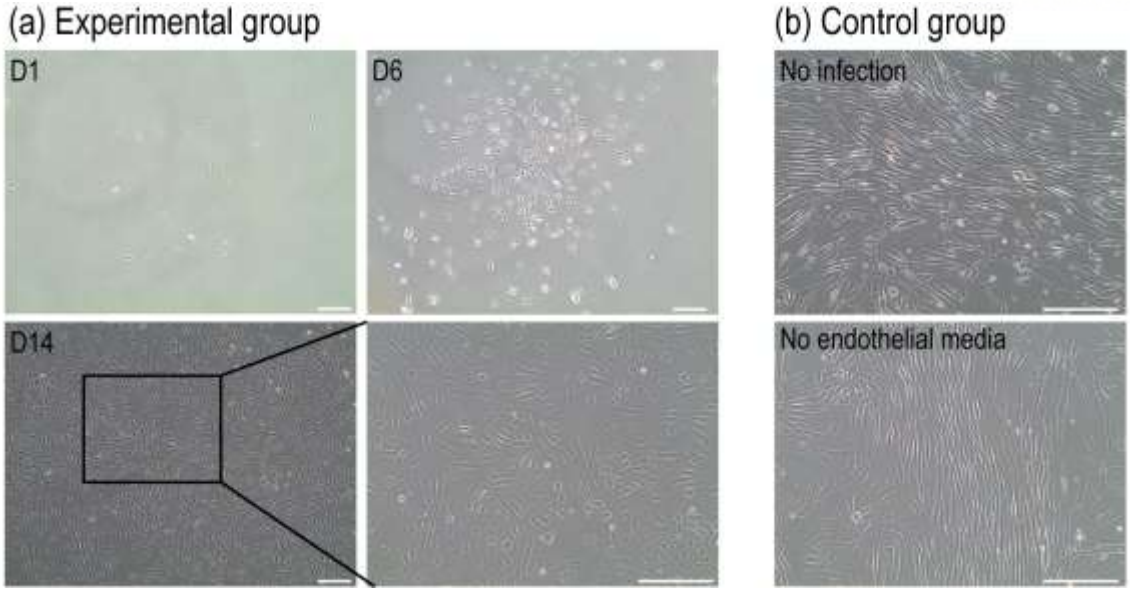


Figure 1.2. Morphological change of fibroblasts into iEndo during direct conversion. (a) Experimental groups are fibroblasts which are infected to *ETV2* lenti-virus and cultured in endothelial media. D1: 1day after *ETV2* infection. D6: 6days after infection and cultured in endothelial induction media for 5days, D14: 14days after infection of the cells. After endothelial induction media was switched into endothelial maintenance media on D6, the cells are cultured in endothelial maintenance media for 8 days. (b) Control groups are included no *ETV2* induction and no endothelial induction media and maintenance media. Scale bars: 250μm

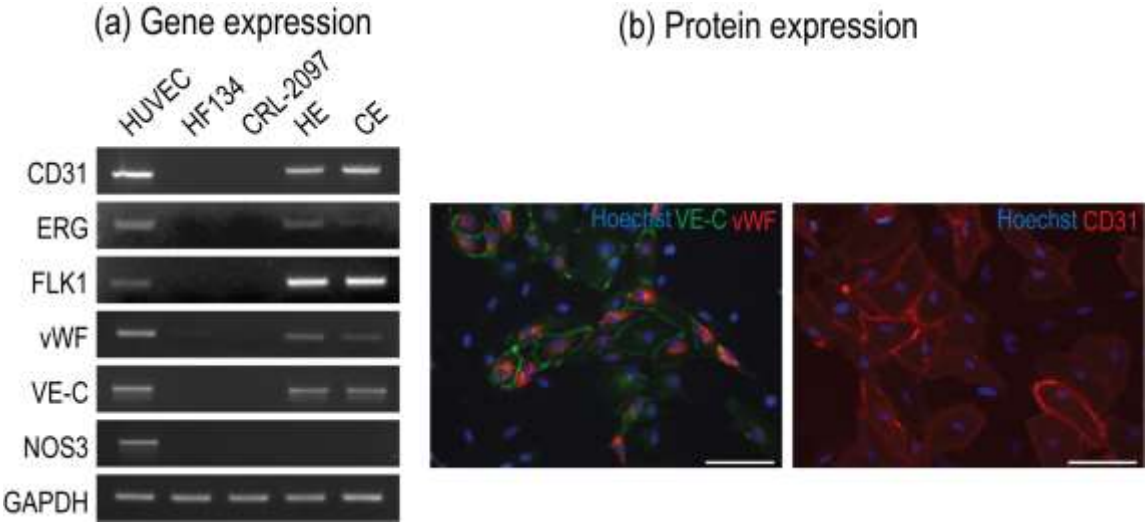


Figure 1.3. Endothelial cell specific marker expressions. (a) Gene expression was analyzed by RT-PCR and (b) protein expression was analyzed by immunocytochemistry. Positive controls: HUVEC (human umbilical vein endothelial cells). Negative controls: parental fibroblasts, HF134 and CRL2097. HE: HF134 infected *ETV2*. CE: CRL2097 infected *ETV2*. Scale bars: 100μm

Figure 1.4. Tubule-structure formation of iEndo. Tubule-structure formation assay iEndo on matrigel-coated dishes 3 days after culture on matrigel. Scale bars: 250μm

2. Screening of endothelial cell specific lncRNA

To find the relationship with vascular specific lncRNA, expression of lncRNAs were also checked. Several lncRNAs reported to be related to vascular endothelial lineage development or function was selected as the candidate lncRNAs. Candidate lncRNAs were screened through RT-PCR amplification from primary endothelial cell (HUVEC) and iEndo cDNA template. Some lncRNAs were specifically expressed in endothelial cells but not in the fibroblasts (Fig.2.1).

Figure 2.1. Screening of candidate lncRNA. Expression of lncRNA which are related to vascular development or function was screened by RT-PCR.

Among them, *SENCR* was focused on the further analysis in this study. To determine the relationship between *SENCR* and *ETV2*, lenti-viral vector and episomal vector was inserted to *SENCR* lncRNA both isoforms for induction (Fig. 2.2). Because *SENCR* have two isoforms, variant 1 and variant 2, both S1 and S2 were amplified with different length. S1 consist of 3 exons with a length of 1060bp. But, S2 consist of 2 exons with a length of 539bp respectively (Fig. 2.3).

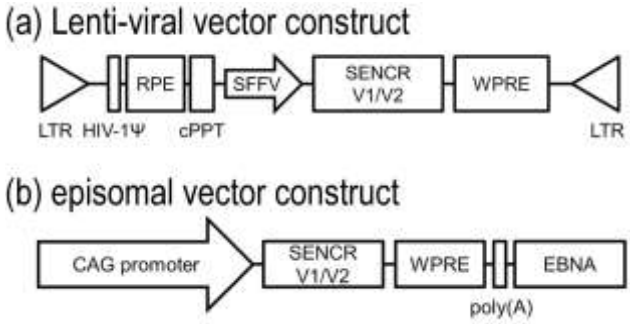
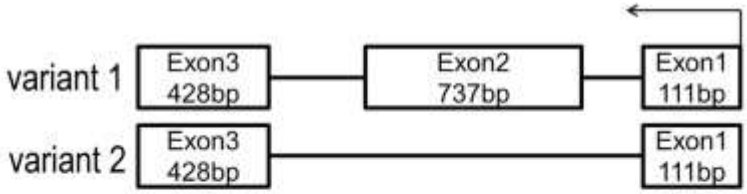


Figure 2.2. Vector constructs for *SENCR* lncRNA induction. (a) Lenti-viral vector constructs encoded *SENCR* variant 1 or 2 (b) Episomal vector constructs encoded *SENCR* variant 1 or 2.

3. ETV2 is regulated by lncRNA SENCR

To determine which vascular specific transcription factor have the potential to be regulated by *SENCR*, two cell lines, HEK293T, and CRL-2097, were infected with *SENCR*. Over-expression of *SENCR* resulted in increase of *ETV2* gene expression in both cell lines. A similar pattern was observed in S1 and S2 (Fig. 3.1a). Also, ETV2 protein expression level was slightly increased in *ETV2* and *SENCR* co-infected group more than *ETV2* single infected groups. However, ETV2 protein expression was not detected when S1 or S2 were over-expressed alone (Fig. 3.1b). Furthermore, *ETV2* promoter activity was estimated using dual-luciferase promoter assay. 1.5kb region of *ETV2* promoter was used for estimation of *ETV2* promoter activity and this sequence was inserted in luciferase promoter reporter vector. When S1 or S2 was overexpressed, *ETV2* promoter activity was enhanced in HEK293T cells dose-dependently. The highest *ETV2* promoter activity was found when 200ng of S2 RNA plasmid was transfected (Fig 3.2). These findings indicate that *SENCR* activates *ETV2* promoter and increases *ETV2* gene and protein expression.

(a)



(b)

>KF806591.1

AGGGCCTGTGCGTAGAGTTTAAGCAGTGTGGAGATATTTCTTCACTTTTCCTTAGGGTGTCC
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CCGCTGCCGAGACGCGGCAGACTCCCAGAGCGGCTCACCCACTCATCGAAGCGGATTCTG
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GCGCTCTGGACCGTCCTCCCTACCCAGCCTGGAGGCCGGCGTTCCCCGGCCCAACCCGG
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CCTCATTCAGCCAGAAAGGACTCCAACTCCACCACCAGGAGGCCCCCCTTCCTGGTGCTG
GCCAGTTGCAGAGGCTGTTCATGGCATCCCACAGGTGGCGACTGCCCCCCACCCTCCTCC
TAGCACTTTAGAGGGTCCCTCTGTGGCAGACACCATGGCTAGGTTTCTCTTTCCAACTCCTG
TTGGTCTGGCAAGTGACAGATCATCTTCACTACATTAATCAGTAGTCACCCGAACATTCCTAT
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>KF806590.1

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GCTCTACCGACCTTCAAACTACAACAGAGCTTTCAGGAGAATGCGGAGAGACGGCTTCCCA
CCCGGACCTCACCAGAAACCCGCACACCCCAGCCACATTGCTCCTCATTCAGCCAGAAAG
GACTCCAACTCCACCACCAGGAGGCCCCCCTTCCTGGTGCTGGCCAGTTGCAGAGGCTGT
TCATGGCATCCCACAGGTGGCGACTGCCCCCCACCCTCCTCCTAGCACTTTAGAGGGTCC
CTCTGTGGCAGACACCATGGCTAGGTTTCTCTTTCCAACTCCTGTTGGTCTGGCAAGTGAC
AGATCATCTTCACTACATTAATCAGTAGTCACCCGAACATTCCTATACTGTCCCCCAATTCCTT
TCAGCACCAGCTGCCTCCCCCACTGGAATTATTCAATATGGAACTAGATTAAATTGAAGACTT
GCCCTTTAGTTTTTAAAAAAAAAAAAAAAAAAAAA

Figure 2.3. *SENCR* gene structure and isoform sequences. (a) Schematic representation of exons of S1 and S2. S1 consist of 3 exons but S2 consist of 2 exons (b) Whole sequence are references form NCBI (S1: KF806591.1, S2: KF806590.1).

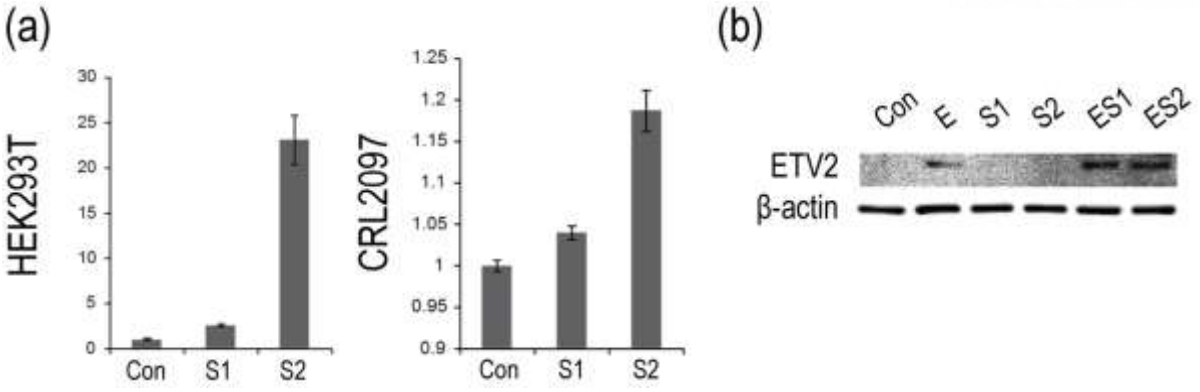

Figure 3.1. *ETV2* gene expression and protein expression after infection of *SENCR*. (a) aRT-PCR analysis of *ETV2* gene expression at 3 days after infection of *SENCR* in HEK293T and CRL2097 cell lines. (b) Western blot analysis of ETV2 protein expression at 3 days after infection of *ETV2*, *SENCR* of *ETV2* and *SENCR* in HEK293T cell lines. Con: no infection, S1: *SENCR* variant 1, S2: *SENCR* variant 2, E: *ETV2*, ES1: *ETV2*+*SENCR* variant1, ES2: *ETV2*+*SENCR* variant 2.

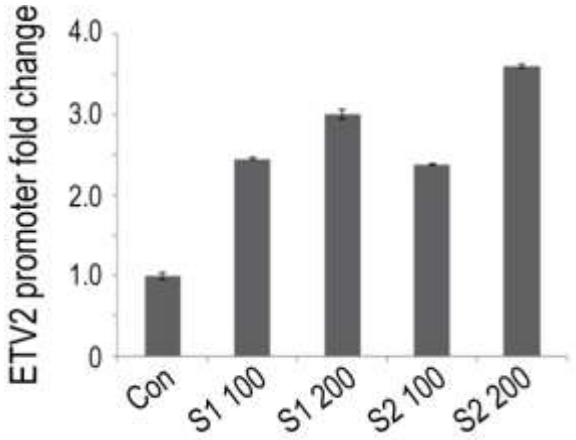

Figure 3.2. Estimation of *ETV2* promoter by dual-luciferase promoter assay. The fold change of *ETV2* promoter activity after overexpression of S1 or S2. Con: no infection, S1: *SENCR* variant 1, S2: *SENCR* variant 2, 100: 100ng, 200 200ng

4. Detection of SENCR binding proteins

To demonstrate the mechanism of *SENCR* in regulation of *ETV2* expression, *SENCR* binding protein candidates were screened. *SENCR* RNAs were successfully synthesized *in vitro* from plasmid encoding *SENCR* gene sequence. Also, anti-sense RNA was generated and used as a negative control (Fig. 4.1a). Through RNA pulldown analysis, some proteins bound to the *SENCR* sense RNA were detected. However, the negative controls, bead-only and antisense RNA, also bound to proteins non-

specifically (Fig. 4.1b). So, additional experiment was needed to distinguish *SENCR* sense RNA specific proteins. The binding proteins were analyzed by shot gun in gel digestion for the LC-MS analysis. Within the proteins identified 33 proteins categorized into the RNA binding proteins in gene ontology (GO) term. Also, the proteins were categories into other ontology categories biological process and cellular component (Fig. 4.2). Also, the results suggest 683 proteins bound to *SENCR* sense RNA only without binding of bead (Fig. 4.3 and 4.4).

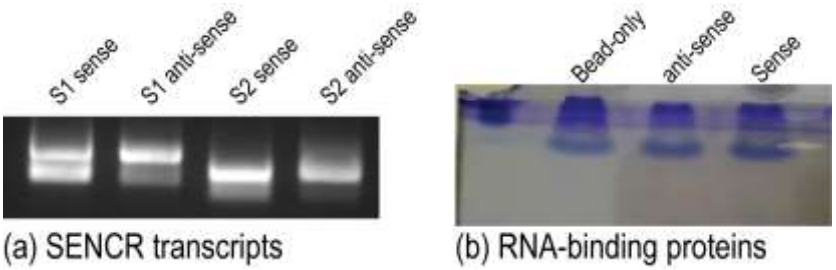

Figure 4.1. *ETV2* gene expression and protein expression after infection of *SENCR*. (a) Gel images of *SENCR* RNA transcripts. S1: *SENCR* variant 1, S2: *SENCR* variant 2 (b) Coomassie blue stained SDS gel image of RNA binding proteins.

5. SENCR recruits PSPC1 to ETV2 promoter for activation

To further investigate how *SENCR* activate *ETV2* gene, protein, and promoter, the candidate proteins were applied to the *ETV2* promoter luciferase assay. Within 53 candidate protein, the rate of PTB-associated proteins and proteins which formed complex with PTB-associated proteins was high. Among them, higher activation of *ETV2* promoter was observed after addition of PSPC1, one of the 53 candidate protein. Only S1 or S2 RNAs activated *ETV2* promoter 1.9- and 1.4-fold respectively in consistence with previous result. Furthermore, co-transfection of PSPC1 with RNAs showed increased *ETV2* promoter activity by 3.9-fold and 3.6-fold when co-transfected with S1 or S2 respectively. However, there was a slight increase in PTBP1, a negative control that was not on the list, transfected group (Fig. 3f). The results indicate that *SENCR* RNAs bind to PSPC1 protein and recruit it to *ETV2* promoter for activation (Fig. 3g).

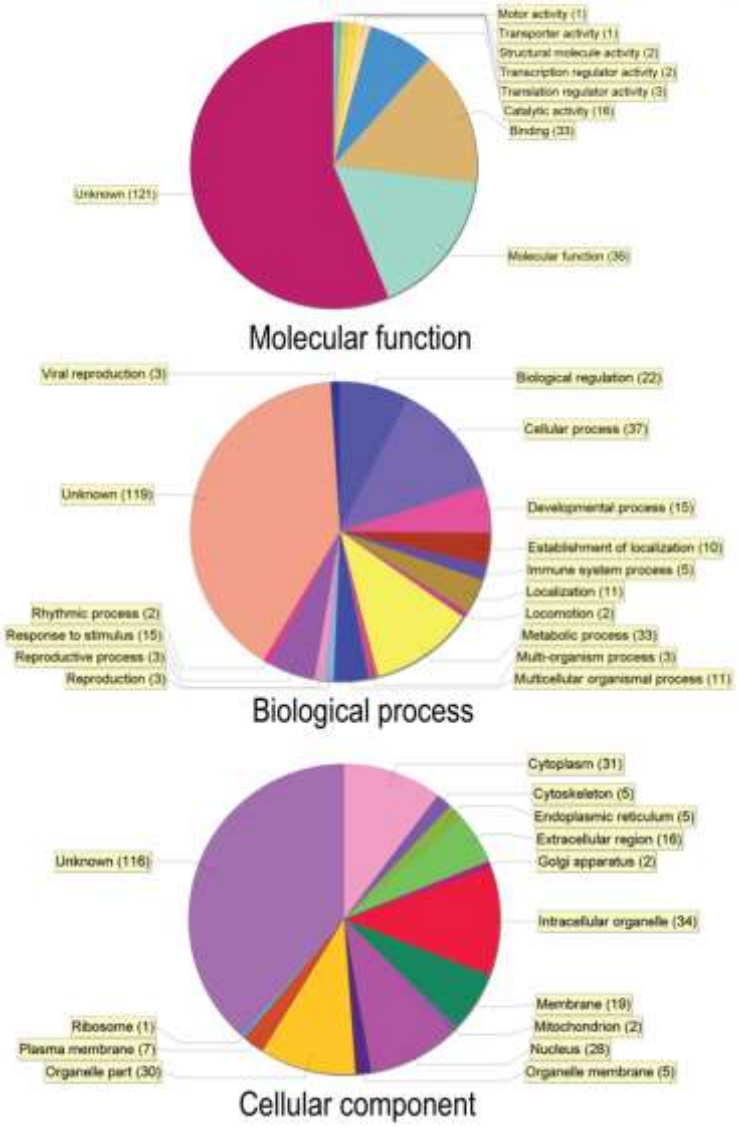

Figure 4.2. GO term analysis of *SENCR* binding proteins. Pie charts indicate *SENCR* binding proteins are analyzed as molecular function, biological process, and cellular component of GO term category.

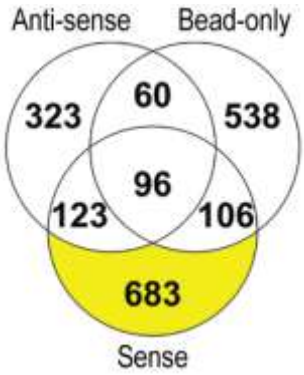

Figure 4.3. The number of RNA-binding proteins. Venn diagram of *SENCR*-binding proteins analyzed by LC-MS. Yellow color indicates the number of *SENCR* sense RNA specific proteins.

Table 4. SENCR RNA binding proteins analyzed by LC-MS

Identified Proteins (157/472)	Accession Number	Molecular Weight	Anti -Sense	Bead	Sense
Splicing factor, proline- and glutamine-rich OS=Homo sapiens GN=SFPQ PE=1 SV=2	SFPQ_HUMAN	76 kDa	1	1	8.00E+08
Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=1	H3BLZ8_HUMAN	80 kDa	#######	1	2.60E+08
ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4	DHX9_HUMAN	141 kDa	4031000	1	1.63E+08
ATP-dependent RNA helicase DHX36 OS=Homo sapiens GN=DHX36 PE=1 SV=2	DHX36_HUMAN	115 kDa	1	1	6.24E+07
Titin OS=Homo sapiens GN=TTN PE=1 SV=1	A0A0A0MTS7_HUMAN	3994 kDa	1	1	9679300
Nucleoporin 153kDa, isoform CRA_a OS=Homo sapiens GN=NUP153 PE=4 SV=1	A0A024QZW7_HUMAN (+1)	154 kDa	819350	1	1473700
ATP-dependent RNA helicase DDX5 (Fragment) OS=Homo sapiens GN=DDX5 PE=2 SV=1	B5BUE6_HUMAN (+2)	69 kDa	1	1	2.89E+08
Junction plakoglobin, isoform CRA_a OS=Homo sapiens GN=JUP PE=4 SV=1	A0A024R1X8_HUMAN (+1)	82 kDa	473220	1	1
Probable ATP-dependent RNA helicase DHX35 OS=Homo sapiens GN=DHX35 PE=1 SV=2	DHX35_HUMAN	79 kDa	#######	1	1.81E+07
Microtubule-associated protein OS=Homo sapiens GN=MAP4 PE=1 SV=1	E7EVA0_HUMAN	245 kDa	790380	1	1
PAX3- and PAX7-binding protein 1 OS=Homo sapiens GN=PAXBP1 PE=1 SV=2	PAXB1_HUMAN	105 kDa	3911500	1	8009100
X-ray repair cross-complementing protein 5 OS=Homo sapiens GN=XRCC5 PE=1 SV=3	XRCC5_HUMAN	83 kDa	1586800	1	2.25E+07
Ubiquitin-activating enzyme E1 (A1S9T and BN75 temperature sensitivity complementing), isoform CRA_a OS=Homo sapiens PE=3 SV=1	A0A024R1A3_HUMAN (+1)	118 kDa	1952900	1	6504300
Ribosomal biogenesis protein LAS1L OS=Homo sapiens GN=LAS1L PE=1 SV=2	LAS1L_HUMAN	83 kDa	7724000	1	4919700
Ribosomal protein L29, isoform CRA_a OS=Homo sapiens GN=RPL29 PE=4 SV=1	A0A024R326_HUMAN (+2)	18 kDa	3082300	1	1
FACT complex subunit SPT16 OS=Homo sapiens GN=SUPT16H PE=1 SV=1	SP16H_HUMAN	120 kDa	1939600	1	6113100
Nucleolin, isoform CRA_b OS=Homo sapiens GN=NCL PE=4 SV=1	A0A024R4A0_HUMAN (+1)	77 kDa	3104300	1	6419200
Heterogeneous nuclear ribonucleoprotein F, isoform CRA_a OS=Homo sapiens GN=HNRPF PE=4 SV=1	A0A024R7T3_HUMAN (+1)	46 kDa	1	1	8.58E+07
X-ray repair complementing defective repair in Chinese hamster cells 6 (Ku autoantigen, 70kDa), isoform CRA_a OS=Homo sapiens GN=XRCC6 PE=4 SV=1	A0A024R1N4_HUMAN (+1)	70 kDa	1	1	1.23E+07
HIV Tat-specific factor 1 OS=Homo sapiens GN=HTATSF1 PE=1 SV=1	HTSF1_HUMAN	86 kDa	1056400	1	1526800
cDNA FLJ51771, highly similar to SWI/SNF-related matrix-associatedactin-dependent regulator of chromatin subfamily A member5 (EC 3.6.1.-) OS=Homo sapiens PE=2 SV=1	B4DZC0_HUMAN (+2)	117 kDa	371380	1	1
cDNA FLJ78483, highly similar to Homo sapiens elongation factor Tu GTP binding domain containing 2 (EFTUD2), mRNA OS=Homo sapiens PE=2 SV=1	A8KAP3_HUMAN (+4)	109 kDa	6959300	1	4686700
Heterogeneous nuclear ribonucleoprotein K, isoform CRA_d OS=Homo sapiens GN=HNRPK PE=4 SV=1	A0A024R228_HUMAN (+4)	51 kDa	#######	1	6149900
ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=1	A0A0D9SF53_HUMAN	81 kDa	1	1	4.09E+07
Guanine nucleotide binding protein-like 3 (Nucleolar)-like, isoform CRA_a OS=Homo sapiens GN=GNL3L PE=4 SV=1	A0A024R9Y6_HUMAN (+2)	66 kDa	#######	1	949750
RPL14 protein OS=Homo sapiens GN=RPL14 PE=1 SV=1	Q6IPH7_HUMAN	24 kDa	723440	1	1
Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase, isoform CRA_a OS=Homo sapiens GN=MTHFD1 PE=3 SV=1	A0A024R652_HUMAN (+3)	102 kDa	2268700	1	1322100
Interleukin enhancer binding factor 3, 90kDa, isoform CRA_d OS=Homo sapiens GN=ILF3 PE=4 SV=1	A0A024R7C7_HUMAN (+5)	95 kDa	1	1	1.84E+07
Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3	DESP_HUMAN	332 kDa	1	1	72198
Cisplatin resistance-associated overexpressed protein, isoform CRA_b OS=Homo sapiens GN=LUC7L3 PE=1 SV=1	J3KPP4_HUMAN (+2)	58 kDa	1	1	2957300
cDNA, FLJ95650, highly similar to Homo sapiens karyopherin (importin) beta 1 (KPNB1), mRNA OS=Homo sapiens PE=2 SV=1	B2RBR9_HUMAN (+1)	97 kDa	4975900	1	3540300
cDNA FLJ56274, highly similar to Transketolase (EC 2.2.1.1) OS=Homo sapiens PE=2 SV=1	B4E022_HUMAN (+3)	63 kDa	#######	1	1
Synaptosomal-associated protein (Fragment) OS=Homo sapiens GN=SNAP23 PE=1 SV=1	H3BV99_HUMAN	16 kDa	1150000	1	1
P37 AUF1 OS=Homo sapiens PE=2 SV=1	Q12771_HUMAN	31 kDa	#######	1	7602700
Nucleolar RNA helicase 2 OS=Homo sapiens GN=DDX21 PE=1 SV=5	DDX21_HUMAN	87 kDa	1	1	3590800
Interleukin enhancer-binding factor 2 OS=Homo sapiens GN=ILF2 PE=1 SV=1	B4DY09_HUMAN (+2)	39 kDa	1226100	1	9465100
Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4	TERA_HUMAN (+1)	89 kDa	2255600	1	2685300
U5 small nuclear ribonucleoprotein 200 kDa helicase OS=Homo sapiens GN=SNRNP200 PE=1 SV=2	U520_HUMAN	245 kDa	1052400	1	1
Proteasome subunit alpha type-5 OS=Homo sapiens GN=PSMA5 PE=1 SV=3	PSA5_HUMAN (+1)	26 kDa	1420100	1	530440
Pre-mRNA-processing factor 40 homolog A OS=Homo sapiens GN=PRPF40A PE=1 SV=2	PR40A_HUMAN	109 kDa	3912900	1	1776500
Profilin (Fragment) OS=Homo sapiens GN=PFN2 PE=3 SV=1	D3DNI2_HUMAN (+1)	35 kDa	1	1	66987
cDNA FLJ60424, highly similar to Junction plakoglobin OS=Homo sapiens PE=2 SV=1	B4DE59_HUMAN	63 kDa	1	1	6.95E+07
39S ribosomal protein L22, mitochondrial OS=Homo sapiens GN=MRPL22 PE=1 SV=1	A0A0C4DGX2_HUMAN (+3)	26 kDa	1266800	1	1
Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens GN=DYNC1H1 PE=1 SV=5	DYHC1_HUMAN	532 kDa	1	1	9990500
A6(IV) collagen (Fragment) OS=Homo sapiens GN=COL4A6 PE=4 SV=1	Q9UEH6_HUMAN	154 kDa	1	1	285280
Chaperonin containing TCP1, subunit 6A (Zeta 1), isoform CRA_a OS=Homo sapiens GN=CCT6A PE=3 SV=1	A0A024RDL1_HUMAN (+5)	58 kDa	4978100	1	1
Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1	ENPL_HUMAN (+2)	92 kDa	#######	1	1.24E+07
Bifunctional glutamate/proline--tRNA ligase OS=Homo sapiens GN=EPRS PE=1 SV=5	SYEP_HUMAN	171 kDa	1041600	1	1
Paraspeckle component 1, isoform CRA_b OS=Homo sapiens GN=PSPC1 PE=4 SV=1	A0A024RDP4_HUMAN (+1)	46 kDa	1	1	2.42E+07
cDNA FLJ78586, highly similar to Homo sapiens VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa (VAPA), mRNA OS=Homo sapiens PE=1 SV=1	A8KA83_HUMAN (+1)	27 kDa	1	1	1064600
Chaperonin containing TCP1, subunit 8 (Theta) variant (Fragment) OS=Homo sapiens PE=2 SV=1	Q53HU0_HUMAN (+2)	60 kDa	9358100	1	2442100
Family with sequence similarity 32, member A, isoform CRA_a OS=Homo sapiens GN=FAM32A PE=4 SV=1	A0A024R7I4_HUMAN (+3)	13 kDa	1	1	216440
Nuclease-sensitive element-binding protein 1 (Fragment) OS=Homo sapiens GN=YBX1 PE=1 SV=1	H0Y449_HUMAN	42 kDa	1946600	1	5254900
Vinculin, isoform CRA_c OS=Homo sapiens GN=VCL PE=4 SV=1	A0A024QZN4_HUMAN (+2)	117 kDa	607030	1	1

Identified Proteins (157/472)	Accession Number	Molecular Weight	Anti -Sense	Bead	Sense
MAP7 domain-containing protein 3 (Fragment) OS=Homo sapiens GN=MAP7D3 PE=1 SV=1	A0A0A0MRP0_HUMAN (+1)	90 kDa	564360	1	1
Coatomer subunit alpha OS=Homo sapiens GN=COPA PE=1 SV=2	COPA_HUMAN	138 kDa	513740	1	1084300
Insulin-like growth factor 2 mRNA binding protein 1 deltaN CRDBP OS=Homo sapiens GN=IGF2BP1 PE=2 SV=1	D3DTW3_HUMAN (+1)	48 kDa	########	1	2954600
Lamina-associated polypeptide 2, isoform alpha OS=Homo sapiens GN=TMPO PE=1 SV=2	LAP2A_HUMAN	75 kDa	4775800	1	4064000
40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=3 SV=1	A0A024R2P0_HUMAN (+3)	33 kDa	8953900	1	3662700
Melanoma antigen family D, 2, isoform CRA_a OS=Homo sapiens GN=MAGED2 PE=4 SV=1	A0A024R9Y7_HUMAN (+2)	65 kDa	2263100	1	2241700
cDNA FLJ54573, highly similar to Importin beta-3 OS=Homo sapiens PE=2 SV=1	B4E0R6_HUMAN (+1)	109 kDa	320350	1	2007500
Tyrosine-protein kinase receptor OS=Homo sapiens GN=KIF5B-ALK PE=2 SV=1	C1PHA2_HUMAN	168 kDa	256490	1	1
Fus-like protein (Fragment) OS=Homo sapiens PE=2 SV=1	Q13344_HUMAN	53 kDa	1	1	8112600
Chaperonin containing TCP1, subunit 7 (Eta) variant (Fragment) OS=Homo sapiens PE=2 SV=1	Q53HV2_HUMAN (+2)	59 kDa	4972600	1	1136800
Dedicator of cytokinesis 4 OS=Homo sapiens GN=DOCK4 PE=4 SV=1	A4D0S8_HUMAN	225 kDa	2960800	1	1
A-kinase anchor protein 8-like OS=Homo sapiens GN=AKAP8L PE=1 SV=3	AKP8L_HUMAN	72 kDa	7539500	1	2814700
Uncharacterized protein OS=Homo sapiens GN=FLJ10154 PE=4 SV=1	A0A024RDW4_HUMAN (+2)	33 kDa	1	1	3296700
cDNA FLJ76877, highly similar to Homo sapiens superkiller viralicidic activity 2-like 2 (SKIV2L2), mRNA OS=Homo sapiens PE=2 SV=1	A8K6I4_HUMAN (+2)	118 kDa	34615	1	1
Eukaryotic translation initiation factor 3 subunit M OS=Homo sapiens GN=EIF3M PE=1 SV=1	EIF3M_HUMAN	43 kDa	52403	1	1
Glucosidase, alpha neutral AB, isoform CRA_b OS=Homo sapiens GN=GANAB PE=3 SV=1	A0A024R592_HUMAN (+8)	96 kDa	275190	1	499540
ADP-sugar pyrophosphatase OS=Homo sapiens GN=NUDT5 PE=1 SV=1	A6NCQ0_HUMAN (+3)	20 kDa	1	1	336350
Acetyltransferase component of pyruvate dehydrogenase complex OS=Homo sapiens PE=2 SV=1	B4DS43_HUMAN	45 kDa	7744800	1	1
Heterogeneous nuclear ribonucleoproteins C1/C2 OS=Homo sapiens GN=HNRNPC PE=1 SV=1	B2R5W2_HUMAN (+4)	32 kDa	5088000	1	2713200
VAMP (Vesicle-associated membrane protein)-associated protein B and C OS=Homo sapiens GN=VAPB PE=1 SV=1	Q53XM7_HUMAN (+1)	27 kDa	753290	1	1182400
Pre-mRNA-processing factor 6 OS=Homo sapiens GN=PRPF6 PE=1 SV=1	PRP6_HUMAN	107 kDa	632900	1	1
Heterogeneous nuclear ribonucleoprotein A3 OS=Homo sapiens GN=HNRNPA3 PE=1 SV=2	ROA3_HUMAN	40 kDa	1	1	1.80E+07
RNA-binding motif protein, X chromosome OS=Homo sapiens GN=RBMX PE=1 SV=3	RBMX_HUMAN	42 kDa	1	1	1808300
cDNA, FLJ94025, highly similar to Homo sapiens tripartite motif-containing 28 (TRIM28), mRNA OS=Homo sapiens PE=2 SV=1	B2R8R5_HUMAN (+1)	89 kDa	501250	1	1
TNF receptor-associated protein 1 variant (Fragment) OS=Homo sapiens PE=2 SV=1	Q53FS6_HUMAN (+1)	80 kDa	########	1	1
cDNA FLJ38393 fis, clone FEBRA2007212 OS=Homo sapiens PE=2 SV=1	B3KTJ9_HUMAN (+1)	103 kDa	588920	1	1160200
Phospholipase DDHD1 OS=Homo sapiens GN=DDHD1 PE=1 SV=2	DDHD1_HUMAN	100 kDa	428350	1	1
Adenosylhomocysteinase OS=Homo sapiens GN=AHCY PE=1 SV=4	SAHH_HUMAN	48 kDa	4883600	1	1
Ribonucleoprotein PTB-binding 1 OS=Homo sapiens GN=RAVER1 PE=1 SV=1	A0A087WZ13_HUMAN (+1)	78 kDa	1	1	587740
keratin type II, KII-9, hair - sheep gi|1308 (X62509) hair type II keratin intermediate filament protein [Ovis aries]	gi|109048|pir||S22025	55 kDa	8967600	1	1
Serine/threonine-protein phosphatase OS=Homo sapiens GN=PPP2CA PE=2 SV=1	Q6I9T8_HUMAN	36 kDa	133080	1	1
Phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase, isoform CRA_c OS=Homo sapiens GN=PAICS PE=3 SV=1	A0A024RD93_HUMAN (+2)	47 kDa	3668800	1	1
DNA ligase 3 OS=Homo sapiens GN=LIG3 PE=1 SV=2	DNLI3_HUMAN	113 kDa	1	1	454830
DEAH (Asp-Glu-Ala-His) box polypeptide 30, isoform CRA_b OS=Homo sapiens GN=DHX30 PE=4 SV=1	A0A024R2T6_HUMAN (+2)	134 kDa	1	1	2046300
Heterogeneous nuclear ribonucleoprotein A/B OS=Homo sapiens GN=HNRNPAB PE=1 SV=1	A0A087WZV1_HUMAN	37 kDa	7465100	1	1
Microfibrillar-associated protein 1 OS=Homo sapiens GN=MFAP1 PE=1 SV=2	MFAP1_HUMAN	52 kDa	7349300	1	1
RNA-binding protein 14 OS=Homo sapiens GN=RBM14 PE=1 SV=2	RBM14_HUMAN	69 kDa	1	1	3838900
Taube nuss homolog (Mouse), isoform CRA_a OS=Homo sapiens GN=TBN PE=4 SV=1	A0A024RD01_HUMAN (+1)	37 kDa	1573700	1	1
Vimentin variant (Fragment) OS=Homo sapiens PE=2 SV=1	Q53HU8_HUMAN (+2)	54 kDa	9971300	1	1.43E+07
DNA-(apurinic or apyrimidinic site) lyase (Fragment) OS=Homo sapiens GN=APEX1 PE=1 SV=1	G3V359_HUMAN (+1)	19 kDa	1162900	1	1
Splicing factor 3B subunit 5 OS=Homo sapiens GN=SF3B5 PE=1 SV=1	SF3B5_HUMAN	10 kDa	33644	1	1
Vesicle-associated membrane protein 2 OS=Homo sapiens GN=VAMP2 PE=4 SV=2	J3QRU4_HUMAN (+2)	12 kDa	1	1	38907
Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3	PDIA1_HUMAN (+2)	57 kDa	1865100	1	1
Exportin-2 OS=Homo sapiens GN=CSE1L PE=1 SV=3	XPO2_HUMAN	110 kDa	1	1	1924900
Cleavage and polyadenylation specificity factor subunit 1 OS=Homo sapiens GN=CPSF1 PE=1 SV=2	CPSF1_HUMAN (+1)	161 kDa	348570	1	1
cDNA, FLJ92954, highly similar to Homo sapiens TAF9-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa (TAF9L), mRNA OS=Homo sapiens PE=2 SV=1	B2R6H6_HUMAN (+1)	28 kDa	1	1	532760
DNA helicase OS=Homo sapiens PE=2 SV=1	B7Z8Z6_HUMAN	107 kDa	276390	1	1
Ribosomal protein L7, isoform CRA_a OS=Homo sapiens GN=RPL7 PE=3 SV=1	A0A024R814_HUMAN (+1)	30 kDa	1	1	490190
60S ribosomal protein L8 (Fragment) OS=Homo sapiens GN=RPL8 PE=1 SV=1	E9PKZ0_HUMAN (+1)	22 kDa	1	1	1569700
40S ribosomal protein S6 OS=Homo sapiens GN=RPS6 PE=2 SV=1	A2A3R6_HUMAN (+2)	29 kDa	1	1	396080
Calmodulin-like protein 5 OS=Homo sapiens GN=CALML5 PE=1 SV=2	CALL5_HUMAN (+1)	16 kDa	385030	1	1
Protein translocation complex beta variant (Fragment) OS=Homo sapiens PE=2 SV=1	Q53FA5_HUMAN (+1)	10 kDa	1	1	143300
MCM10 minichromosome maintenance deficient 10 (S. cerevisiae), isoform CRA_b OS=Homo sapiens GN=MCM10 PE=1 SV=1	Q5T670_HUMAN (+1)	96 kDa	209350	1	1
Myosin, heavy polypeptide 9, non-muscle, isoform CRA_a OS=Homo sapiens GN=MYH9 PE=4 SV=1	A0A024R1N1_HUMAN (+1)	227 kDa	871270	1	1

Identified Proteins (157/472)	Accession Number	Molecular Weight	Anti -Sense	Bead	Sense
Heterogeneous nuclear ribonucleoprotein H2 OS=Homo sapiens GN=HNRNPH2 PE=1 SV=1	HNRH2_HUMAN	49 kDa	1	1	1.59E+08
HCG1990625, isoform CRA_a OS=Homo sapiens GN=hCG_1990625 PE=4 SV=1	D6W507_HUMAN	17 kDa	2159400	1	1
U2 small nuclear RNA auxillary factor 1 isoform a OS=Homo sapiens GN=U2AF1 PE=2 SV=1	B5BU08_HUMAN (+1)	28 kDa	1	1	1.39E+07
KERATIN, HIGH-SULFUR MATRIX PROTEIN, IIIA3 gi|71384|pir||KRSHA3 keratin high-sulfur matrix protein IIIA3 - sheep	gi|125644|sp|P02441|KRA3_SHEEP	14 kDa	995020	1	1
cDNA FLJ55895, highly similar to Ras-related protein Rab-7 OS=Homo sapiens PE=2 SV=1	B4DPH9_HUMAN (+4)	22 kDa	609410	1	61476
Ribosomal protein L34, isoform CRA_a OS=Homo sapiens GN=RPL34 PE=4 SV=1	A0A024RDH8_HUMAN (+1)	13 kDa	1	1	102810
CTP synthase 1 OS=Homo sapiens GN=CTPS1 PE=1 SV=2	PYRG1_HUMAN	67 kDa	3831400	1	1
Eukaryotic translation initiation factor 6 OS=Homo sapiens GN=EIF6 PE=1 SV=1	IF6_HUMAN	27 kDa	583180	1	1
cDNA FLJ50510, highly similar to Heat shock 70 kDa protein 4 OS=Homo sapiens PE=2 SV=1	B4DH02_HUMAN (+2)	94 kDa	1	1	528900
DNA helicase (Fragment) OS=Homo sapiens GN=RUVBL1 PE=2 SV=1	B5BUB1_HUMAN (+1)	50 kDa	1569700	1	1
Nucleosome assembly protein 1-like 1, isoform CRA_a OS=Homo sapiens GN=NAP1L1 PE=3 SV=1	A0A024RBB7_HUMAN (+9)	45 kDa	1910000	1	1
Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6 PE=1 SV=1	PDIA6_HUMAN	48 kDa	2498100	1	1
Structural maintenance of chromosomes protein OS=Homo sapiens GN=SMC2L1 PE=3 SV=1	A0A024R158_HUMAN (+4)	136 kDa	277990	1	1
THO complex subunit 2 OS=Homo sapiens GN=THOC2 PE=1 SV=2	THOC2_HUMAN	183 kDa	1	1	739410
Putative ATP-dependent RNA helicase DHX57 OS=Homo sapiens GN=DHX57 PE=1 SV=2	DHX57_HUMAN	156 kDa	1	1	4413900
Dihydrolipoyl dehydrogenase OS=Homo sapiens GN=DLD PE=4 SV=1	A0A024R713_HUMAN (+4)	54 kDa	3826000	1	1
Ubiquitin-conjugating enzyme E2L 3, isoform CRA_a OS=Homo sapiens GN=UBE2L3 PE=3 SV=1	A0A024R1A4_HUMAN (+2)	18 kDa	239370	1	1
Inosine-5'-monophosphate dehydrogenase 2 (Fragment) OS=Homo sapiens GN=IMPDH2 PE=1 SV=1	H0Y4R1_HUMAN (+1)	51 kDa	1	1	2184000
60S ribosomal protein L13 OS=Homo sapiens GN=RPL13 PE=2 SV=1	A8K4C8_HUMAN (+2)	24 kDa	1	1	729750
Dynein heavy chain 2, axonemal OS=Homo sapiens GN=DNAH2 PE=2 SV=3	DYH2_HUMAN	508 kDa	1	1	1252700
Golgin subfamily A member 8J OS=Homo sapiens GN=GOLGA8J PE=3 SV=3	GOG8J_HUMAN (+1)	72 kDa	1	1	4571300
Proteasome subunit alpha type (Fragment) OS=Homo sapiens GN=PSMA4 PE=1 SV=1	H0YL69_HUMAN (+2)	26 kDa	1	1	1115500
60S ribosomal protein L6 OS=Homo sapiens GN=RPL6 PE=2 SV=1	Q8TBK5_HUMAN	33 kDa	219100	1	1
CAD protein OS=Homo sapiens GN=CAD PE=1 SV=1	F8VPD4_HUMAN (+1)	236 kDa	330180	1	1
Capping protein (Actin filament) muscle Z-line, beta, isoform CRA_a OS=Homo sapiens GN=CAPZB PE=1 SV=1	B1AK87_HUMAN (+4)	29 kDa	1024900	1	239710
28S ribosomal protein S34, mitochondrial OS=Homo sapiens GN=MRPS34 PE=1 SV=2	C9JJ19_HUMAN (+1)	26 kDa	1	1	695200
Uncharacterized protein OS=Homo sapiens GN=MGC20255 PE=4 SV=1	A0A024R0L2_HUMAN (+1)	45 kDa	1	1	1759700
cDNA FLJ78309, highly similar to Homo sapiens heterogeneous nuclear ribonucleoprotein U-like 1 (HNRPUL1), transcript variant 1, mRNA OS=Homo sapiens PE=2 SV=1	A8K5K0_HUMAN (+2)	96 kDa	1	1	1611000
Aspartate aminotransferase OS=Homo sapiens GN=GOT2 PE=3 SV=1	A0A024R6W0_HUMAN (+3)	47 kDa	2394100	1	1
cDNA FLJ53176, highly similar to Nuclear autoantigenic sperm protein OS=Homo sapiens PE=2 SV=1	B4DS57_HUMAN (+4)	75 kDa	413040	1	1
RAB6A, member RAS oncogene family, isoform CRA_b OS=Homo sapiens GN=RAB6A PE=3 SV=1	A0A024R5H8_HUMAN (+3)	24 kDa	730210	1	1
PEST proteolytic signal-containing nuclear protein OS=Homo sapiens GN=PCNP PE=1 SV=2	PCNP_HUMAN	19 kDa	387070	1	1
Protein disulfide-isomerase A4 OS=Homo sapiens GN=ERP70 PE=3 SV=1	A0A090N8Y2_HUMAN (+1)	73 kDa	640850	1	1
Eukaryotic translation initiation factor 3 subunit I OS=Homo sapiens GN=EIF3I PE=1 SV=1	EIF3I_HUMAN (+2)	37 kDa	934180	1	1
Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2	CALX_HUMAN	68 kDa	1	1	1522700
Guanine nucleotide binding protein-like 3 (Nucleolar), isoform CRA_b OS=Homo sapiens GN=GNL3 PE=4 SV=1	A0A024R2Z6_HUMAN (+1)	61 kDa	2463400	1	1
RuvB-like 2 OS=Homo sapiens GN=RUVBL2 PE=1 SV=3	RUVB2_HUMAN	51 kDa	1126200	1	1
Serologically defined breast cancer antigen NY-BR-87 (Fragment) OS=Homo sapiens PE=2 SV=1	Q9H273_HUMAN	20 kDa	462620	1	1
RNA-binding protein 26 OS=Homo sapiens GN=RBM26 PE=1 SV=1	A0A087X0H9_HUMAN (+1)	114 kDa	129110	1	1
TAF6-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa, isoform CRA_a OS=Homo sapiens GN=TAF6L PE=4 SV=1	A0A024R5A7_HUMAN (+1)	68 kDa	2963200	1	1
Heterogeneous nuclear ribonucleoprotein H3 (2H9), isoform CRA_a OS=Homo sapiens GN=HNRPH3 PE=4 SV=1	A0A024QZK8_HUMAN (+1)	37 kDa	1	1	4081700
DnaJ (Hsp40) homolog, subfamily C, member 13 OS=Homo sapiens GN=DNAJC13 PE=2 SV=1	A7E2Y5_HUMAN (+1)	254 kDa	229360	1	1
Alanine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=AARS PE=1 SV=2	SYAC_HUMAN	107 kDa	298830	1	1
Ig heavy chain V-III region GAL OS=Homo sapiens PE=1 SV=1	HV320_HUMAN	13 kDa	1	1	11728
Far upstream element-binding protein 2 OS=Homo sapiens GN=KHSRP PE=1 SV=1	A0A087WTP3_HUMAN (+2)	73 kDa	1	1	1127900
U2 small nuclear RNA auxiliary factor 2 isoform b OS=Homo sapiens GN=U2AF2 PE=2 SV=1	B5BU25_HUMAN (+2)	53 kDa	1	1	2130600
Reticulon OS=Homo sapiens PE=2 SV=1	B7Z3E3_HUMAN (+2)	101 kDa	1354100	1	1
SET translocation (Myeloid leukemia-associated), isoform CRA_b OS=Homo sapiens GN=SET PE=3 SV=1	A0A024R895_HUMAN (+6)	32 kDa	1402600	1	1
IQ motif and SEC7 domain-containing protein 1 OS=Homo sapiens GN=IQSEC1 PE=1 SV=1	A0A087WWK8_HUMAN (+3)	124 kDa	48447	1	1

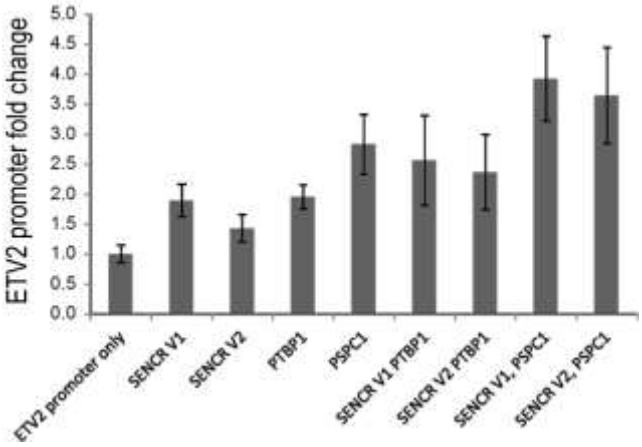

Figure 5.1. Estimation of *ETV2* promoter by dual-luciferase promoter assay. The fold change of *ETV2* promoter activity after overexpression of *SENCR*, binding protein or *SENCR* and binding protein.

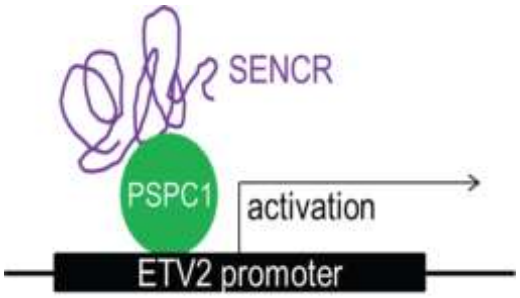

Figure 5.2. Schematic mechanism of *ETV2* regulated by *SENC*R and PSPC1. *SENCR* is seemed to recruit PSPC1 protein to ETV2 promoter for activation.

IV. Discussion

Recent direct conversion studies of endothelial cells have revealed the importance of the ETV2 gene in generation of induced endothelial cells[95-98]. In this study, human fibroblasts switched cell fate into endothelial cells by inducing lentiviral vector encoding single transcription factor *ETV2*. In previous study, generation of iEndo by induction of *ETV2* results from synergistic interaction between *ETV2* and *FOXC2*. Endogenous expression of the *FOXC2* in fibroblast coordinate with exogenously introduced *ETV2* through a composite DNA-binding site, the FOX:ETS motif. Conserved FOX:ETS motif is identified as numerous endothelial enhancer and promoters[87]. Moreover, knockdown of *FOXC2* in fibroblasts resulted in not only significantly reduced *ERG* and *FLI1* expression after *ETV2* infection, but also markedly suppressed endothelial induction[98]. Consistent with previous studies, this report demonstrate that *ETV2* single factor has enough power to generate iEndo from two fibroblast cell lines, CRL-2097 and HF134.

After endothelial induction, some population showed cobble-stone like morphologies but other population remained spindle shape. To increase the efficiency of endothelial conversion, two step endothelial induction protocol was developed in this study by modifying the previous protocol[96-98]. In this study, the efficiency of endothelial cell generation was up to 29.4% (data not shown), which higher efficiency than the previous studies (4~20%) [96-98]. iEndo also showed endothelial lineage gene expression, protein expression and tubule formation function. However, the expression of *NOS3* gene was not detected. Although mouse iEndo generated by direct conversion from fibroblast successfully produced nitric oxide (NO) [97], the human *NOS3* gene has never been expressed in other studies [95-96, 98]. Production of NO by *NOS3* is important *in vivo* because the endothelium plays a central role in regulating smooth muscle tone[121], blood pressure[122-123] , plasma levels of cholesterol[124-125] and maintaining homeostasis[123, 126] through the production of NO by *NOS3* which is regulated by mechanical stimuli, cytokines and growth factors. Therefore, a further study to discover additional transcription factors or stimuli for acquirement of *NOS3* function in iEndo is needed. Another function of endothelial cell is tubular structure formation. iEndo successfully formed capillary structure that means iEndo is considered as functional endothelial cells.

iEndo also expressed vascular specific lncRNAs similar to HUVEC. Among the lncRNAs, we focused on *SENCR*. *SENCR* was identified as vascular cells, endothelial cell and smooth muscle cell, regulator[118, 120]. Also, *SENCR* plays a role of commitment and function during human ESCs differentiation into endothelial cells[119]. Regardless of the overlap of the chromosomal location of *SENCR* and *FLI1* gene, the influence of *SENCR* modulation on *FLI* expression and vice versa has not yet been described. Boulberdaa *et al*. demonstrated that two genes were irrelevant through gain- and loss-of-function experiment in endothelial cells[119]. Therefore, we anticipated that there would be a vascular transcription factor that is regulated by *SENCR*. Since *ETV2* was found to be a strong

transcription factor in vascular formation, we examined whether there was a interaction between *SENCR* and *ETV2*. In this report, *SENCR* was identified as an activator of *ETV2* through recruitment of PSPC1 protein to *ETV2* promoter. This is the first study demonstrates *ETV2* activation by lncRNA and their binding protein. PSPC1 was first identified as a structural protein of the subnuclear structure called the paraspeckle[127]. Previous studies found PSPC1 have capacity to bind other noncoding RNAs[128-129]. This protein have important role in other mesoderm lineage cell such as RNA maturation in adipogenesis[130] and regulation of androgen receptor(AR)-mediated transcriptional activity in spermatogenesis[131]. AR is a ligand-inducible transcription factor. Expression of AR in vascular cells was identified in previous reports[132]. Therefore, it may be expected that there is a correlation of AR mediated endothelial transcription regulation through lncRNA.

Upon vascular development, expression of *ETV2* was consistently increased on the first day of differentiation and silenced in fully differentiated endothelial cells. *SENCR* expression was not dependent on *ETV2* silencing at the end of differentiation. This asks the question of how *SENCR* remained expressed as well in endothelial cell and raises the need for further experimentation to reveal other related mechanisms.

V. Reference

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Acknowledgement

Because many people cheered up me, I was able to work hard for my experiment. I am grateful for the support of everyone who helped to complete this paper.

I would like to appreciate to my supervisor, Professor Jeong Beom Kim, for accepting me as a master's degree graduate student. This thesis was supervised by Professor Jeong Beom Kim. Experimental guidance and teaching which I was given during graduation course will not be forgotten. Also, I would like to appreciate to my thesis examining committee, professor Chan Young Park and professor Tae Joo Park. I learned many things from their advice and comment at my thesis defense presentation.

I have received various advice and support from my colleagues. Firstly, I would like to appreciate to Postdoctoral fellows, Dr. Sang Min Lee who designed and led the direction of lncRNA mechanism study with me and taught molecular experimental technique to me and Dr. Seok Jin Lee who taught us what graduate student should learn basically. Also, I want to express my gratitude to my laboratory senior students. Thanks to Myung Rae Park, Hyunah Lee and Soo Young Park who contributed to set up experiments about generation and characterization of endothelial cells. Your diverse advices have greatly influenced me to finish my degree. Also, thanks to Dong Gyu Nam who taught writing thesis from start to end and discussed about presentation with me. You will soon become a wonderful scientist because you do your best in everything. And, thanks to Hong Dae Seo and Ji Hoon Son who are junior students in this laboratory. I could not help you much, but I hope that your passion for studying will last. And, thanks to my friends who entered at the same time, Ji-hye Park and Juchan Lim. I believe we can succeed on our own field and meet again. Additionally, thanks to calcium dynamics laboratory students who helped setting up protein works and luciferase assay experiments. Thank you for helping the experiments in the middle of busy days.

Lastly, I want to share my accomplishment with big supporters, my mother and sister. The unstinted love of my family made me stronger. Thank you for always cheering me so that I could do my best in my study.

The authors declare no conflict of interest.