HECAT5 regulates cell proliferation via interaction with M3R in cell type-specific manner

by

Chang Sung Woo

Major in Neuroscience

Department of Biomedical Sciences

The Graduate School, Ajou University

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Supervised by

Myung Ae Lee, Ph.D.

Major in Neuroscience

Department of Biomedical Sciences

The Graduate School, Ajou University

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This certifies that the dissertation

of Chang Sung Woo is approved.

SUPERVISORY COMMITTEE

_____

Myung Ae Lee

_____

Young Bae Kim

_____

Jae Ho Lee

The Graduate School, Ajou University

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- ABSTRACT -

HECAT5 regulates cell proliferation via interaction with M3R in cell type-specific manner

Embryonic stem (ES) cells are derived from pluripotent inner cell mass of the early mammalian embryo. Their self-renewal and pluripotency make them attractive sources for stem cell therapies. For ethical and practical reasons, ES cells have a major threat limiting in potential therapeutic applications. To investigate the mechanism regulating stemness, especially cell cycle of ES cells, our previous study identified HECAT5 which is co-expressed in both ES and cancer cells, but not normal tissues by using Digital Differential Display (DDD).

Overexpression of HECAT5 promotes self-renewal capacity and it enhances tumorigenicity in cell type-specific manner. HECAT5 has effects on those through the interaction with M3R (Muscarinic acetylcholine receptor 3), candidate interacting partner of HECAT5, as modulators of M3R activities regulate cell proliferation in a HECAT5-dependent manner. In addition, overexpressed HECAT5 have different effects on the phosphorylation level of M3R in each cell types. In F3 cells, phosphorylation level of M3R was relatively low but HECAT5 enhanced its level. Meanwhile, in HEK293 cells, basically its level was high but HECAT5 decreased it. Besides, F3 and HEK293 cells showed different signaling pathways, ERK and AKT. Collectively, our study uncovers that a novel HECAT5 gene may play a role in regulating the stemness properties in cell type-specific manner.

Key word : Embryonic stem cell, Self-renewal, Pluripotency, Cell proliferation, Stemness

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ABBREVIATION

hESC : human embryonic stem cell

iPSC : induced pluripotent stem cell

DDD : digital differential display

M3R : muscarinic acetylcholine receptor 3

Ⅰ. INTRODUCTION

Embryonic stem (ES) cells are primitive cells with the capacity to divide and lead to more alike stem cells or to form specific cells of somatic tissues. All ES cells are capable of self-renewal, when they divide one daughter cell will be an alike copy of the parent and will remain a stem cell, the other daughter cell may also be alike or may be turned over to differentiate, pluripotent cells. That is they can lead to tissues from all three dermal layers (ectoderm, mesoderm and endoderm) and therefore make cells of any type in the body (J. C. Mountford et al, 2008). Despite the prospective benefit of using ES cells in the regenerative medicine, theirs use remains debatable because of their derivation from early embryos (Guido de Wert et al, 2003). Besides, the issues of potential immunologic rejection and tumorigenicity formation continue to be challenges to clinical cell transplantation approaches. The recent finding of human induced pluripotent stem cells (hiPSCs) has resolved some of these concerns because hiPSCs can be generated autologously and do not require the destruction of embryos (Kamileh Narsinh et al, 2011). Induced pluripotent stem cells (iPSCs) were first discovered via the ectopic expression of four defined factor genes, Oct3/4, Sox2, c-Myc, and Klf4. However, adverse effects of c-Myc introduction into iPSCs have been found. For example, various cancers arise in chimeric mice developed from iPSCs, in which the silencing of exogenous genes usually occurs and the reactivation of the c-Myc oncogene is certain (Ryoko Araki et al, 2011). These observations led us to discover

safe gene which can be substitution source of c-Myc in iPSC generation.

One of the most significant issues in hESC is understanding the mechanisms that modulate stemness of hESC. In this study, we aimed to clarify stemness factor especially regulating self-renewal capacity in hESC using a gene expression profiling approach. By using DDD, we identified genes which are co-expressed in both hES and cancer cells, but not normal tissues.

Ⅱ. MATERIAL AND METHODS

A. Cells and Cell culture

Three cell lines, HB1.F3 (immortalized human neural stem cells), NIH3T3 (mouse embryonic fibroblast cells), HEK293 (human embryonic kidney cell) were cultured in Dulbecco's modified Eagle's medium (DMEM, sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Hyclone), 10ug/ml penicillin-streptomycin (Gibco) in a humidified atmosphere containing 5% CO_2 at 37℃.

B. RT-PCR

Total cell RNAs were extracted using TRIzol reagent (Invitrogen) and the reverse transcription reaction was performed using a Superscript II (Invitrogen) and oligo(dT) (Invitrogen). The 'human M3R' transcripts were amplified by polymerase chain reaction (PCR) in a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) using the primers: forward: 5′-ACG CTC CAA CAG GAG GAA GTA TGG CCG CTG-3′ and reverse: 5′-GAA GTC TTA GCT GTG TCC ACG GCT GAC TC-3′, and the amplified fragment was 420 bp in length. The thermal profile was as follows: 94°C for 5 min; 29 cycles with denaturation at 94°C for 45 sec, annealing at 61°C for 45 sec, and extension at 72°C for 45 sec; with a final extension at 72°C for 10 min. The 'mouse M3R' transcripts were amplified using the primers : forward: 5′-GCA GCT ATG AGC TAC AAC AGC AAG GCA CG-3′ and reverse: 5′-AGG TCT TGG CTG TGT CCA CGG CAG ACT C-3′. The amplified fragment was 450 bp in length, and the thermal profile was as follows: Denaturation at 94°C

for 45 sec, annealing at 63.8°C for 45 sec, and extension at 72°C for 45 sec for 29 cycles, with a final extension at 72°C for 10 min. The PCR products were then subjected to 1% agarose gel electrophoresis with TAE buffer.

C. Membrane preparation and western blotting

Cells were washed and sonicated in ice-cold 20 mM HEPES solution containing 1 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiothreitol, protease inhibitor cocktail (Roche), phosphatase inhibitor cocktail (Roche) (3×30 s, Branson Sonifer). The samples were centrifuged at 600×g at 4°C, and the supernatants were further centrifuged at 20,000×g at 4°C. The pellets (P2 membrane fractions) were subjected to SDS/PAGE (50μg of membrane protein) and immunoblotting. Protein concentration was measured by the Bio-Rad DC Protein Assay. The protein samples were transferred to polyvinylidene difluoride membranes (0.45 μm, Millipore) and probed with monoclonal antibody specific for M3R (Abcam, Cambridge, UK) at a dilution of 1:2500. HRP-conjugated anti-rabbit was used as secondary antibodies (Cell signaling), followed by ECL detection of the corresponding protein bands.

D. ERK/AKT Western blot analysis

Cells were lysed with lysis buffer: RIPA lysis buffer [1% sodium deoxycholoate, 1% triton X-100, 0.1%SDS, 50mM Tris HCl, 50mM NaCl in distilled water, (pH 7.5)] containing protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche) used for extraction of most proteins. Protein concentration was measured by the Bio-Rad DC Protein Assay. The protein samples (30ug) were transferred to polyvinylidene difluoride membranes

(0.45μm, Millipore) and probed with polyclonal antibody specific for total ERK, p-ERK, total AKT, p-ATK(Cell signaling) at a dilution of 1:2500. HRP-conjugated anti-rabbit was used as secondary antibodies (Cell signaling), followed by ECL detection of the corresponding protein bands.

E. Cell Proliferation Assays

Anchorage-dependent cell proliferation was measured using Countess automated cell counter (Thermo Fisher Scientific) and hemocytometer manual cell counting. Anchorage-independent cell proliferation was determined by a soft agar assay. Cells were seeded at a density of $5x10^4$cells per $35mm^3$ cell culture dish in 0.35% agar and cultured for 4 weeks at 37°C under 5% CO2. Dishes were stained with 0.1% crystal violet overnight at 4°C. Colonies were counted and the colony size was determined by a microcaliper.

F. Neurosphere assay

Neurosphere were generated by culturing control and HECAT5 overexpressed cells in DMEM/F12 (Hyclone) supplemented with 2% B27 (GIBCO), 8 mM HEPES (GIBCO) , 1% penicillin-streptomycin (Hyclone) and 20 ng/mL of EGF and bFGF (R&D systems) for 11 days.

G. In Vivo Tumor Growth

Six week old, female, athymic BALB/c Nude mice were purchased from Orient Bio (Kyunggi-do). The mice were maintained in accordance with institutional guidelines, Ajou

University. HECAT5 overexpressed HB1.F3, HEK293 were injected subcutaneously (1 x 10^6 , 5 x 10^6 cells in 0.05ml PBS per animal) with a 27-gauge needle into the both side lower flanks of mice. The tumor growth was measured every second day. The tumor volumes were measured with calipers and calculated with the standard formula of (width^2 × length)/2 where length is the largest measurement. Tumor development was monitored for 4 weeks. When the tumor size reached 2 cm^3, mice were killed.

Ⅲ. RESULTS

A. HECAT5 overexpression increases neurosphere formation in cell type-specific manner.

One of the most important properties of stemness is self-renewal. To ascertain the role of the HECAT5 in the self-renewal, we analyzed sphere formation ability of HECAT5 overexpressed cell lines. Control and HECAT5 overexpressed cells were seeded in 35mm^3 ultra-low attachment dish at a density of 5×10^3cells/ml in DMEM/F12 medium supplemented with B27, EGF, and bFGF. After culturing for 11 days, HECAT5 overexpressed F3, HEK293 cells exhibited a marked ability for sphere formation, compared with control cells. In addition, HECAT5 promoted anchorage-independent growth, indicating that the HECAT5 plays an important role in the maintenance of sphere growth. But, HECAT5 does not effect on sphere formation in NIH3T3 cell. These results suggested that HECAT5 overexpressed cells possess stem-like properties in cell type-specific manner.

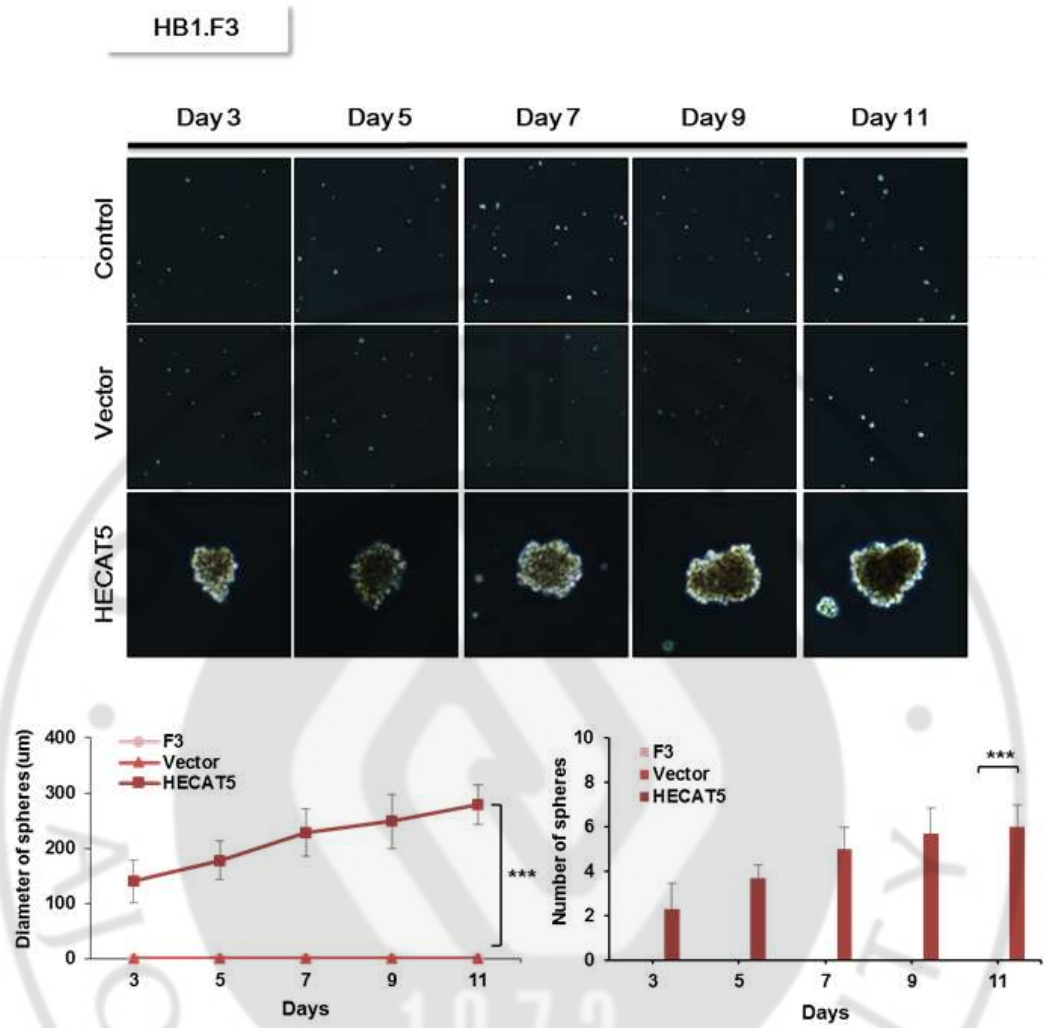


Fig. 1a. HECAT5 overexpression increases neurosphere formation. Neurosphere formation between F3 and F3-HECAT5 was compared on various days. Diameter and Number of spheres were measured and the average values were graphically presented. The data show that HECAT5 increases the number and size of neurospheres.

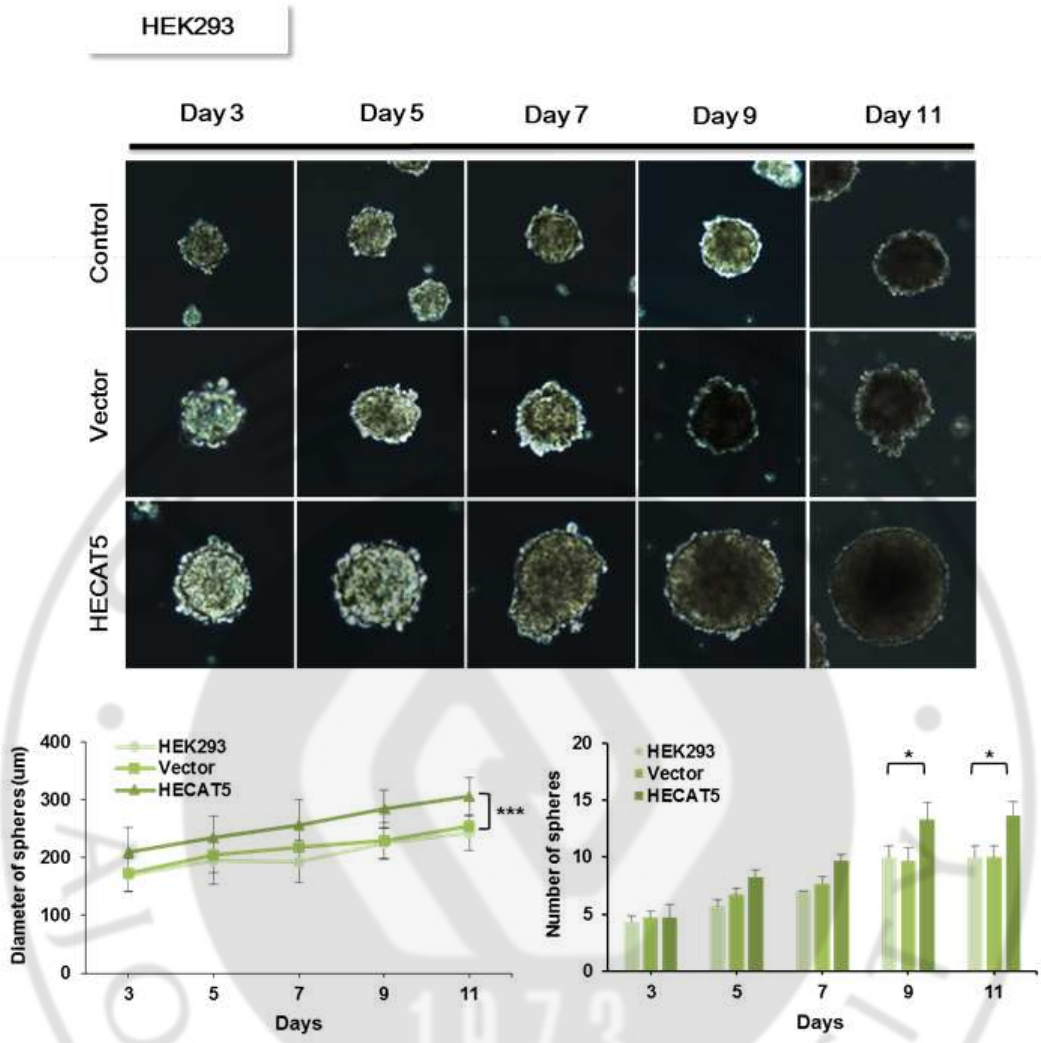


Fig. 1b. HECAT5 overexpression increases neurosphere formation. Neurosphere formation between HEK293 and HEK-HECAT5 was compared on various days. Diameter and Number of spheres were measured and the average values were graphically presented. The data show that HECAT5 increases the number and size of neurospheres.

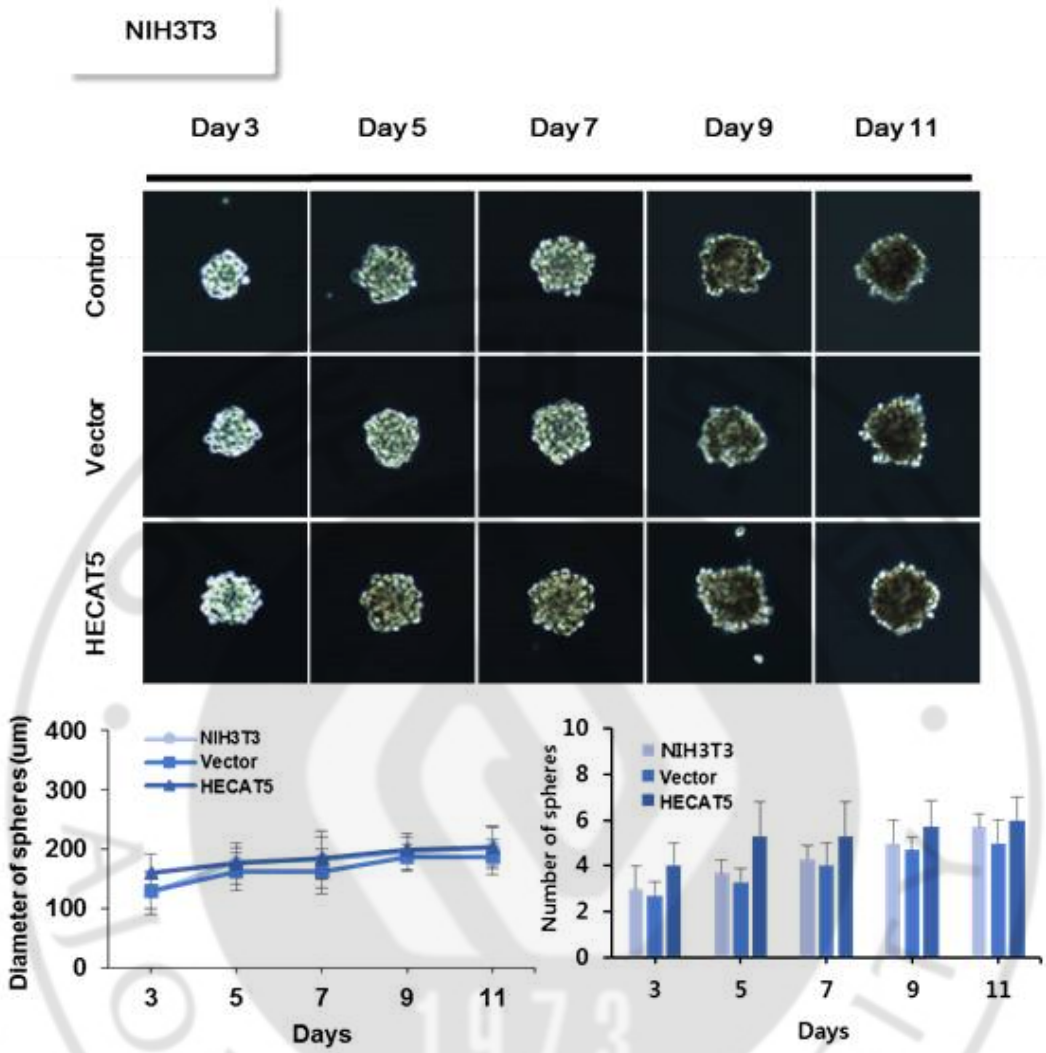


Fig. 1c. HECAT5 overexpression dose not effect on neurosphere formation. Neurosphere formation between NIH3T3 and NIH-HECAT5 was compared on various days. Diameter and Number of spheres were measured and the average values were graphically presented. The data show that HECAT5 does not effect on the number and size of neurospheres in NIH3T3 cell.

B. Overexpression of HECAT5 increases tumorigenicity in cell type-specific manner.

The anchorage-independent growth of HECAT5 overexpressed cell was analyzed by a colony formation assay in soft agar (Figure 2A). HECAT5 increased colony number by 20 folds, from 130.6 ± 54.4 to 2148.8 ± 296.6 in F3 cell and 50 folds, from 47.5 ± 20.6 to 2778 ± 268.9 in HEK293 cell (Figure 2B). The colony size was also increased from an average diameter of 3.4 ± 1.4 to 31.7 ± 10.5 μm in F3 cell and from 3 ± 1.3 to 37.2 ± 10.8 μm in HEK293 cell (Figure 2C). These results indicate a specific role for HECAT5 in anchorage-independent growth of HECAT5 overexpressed cells.

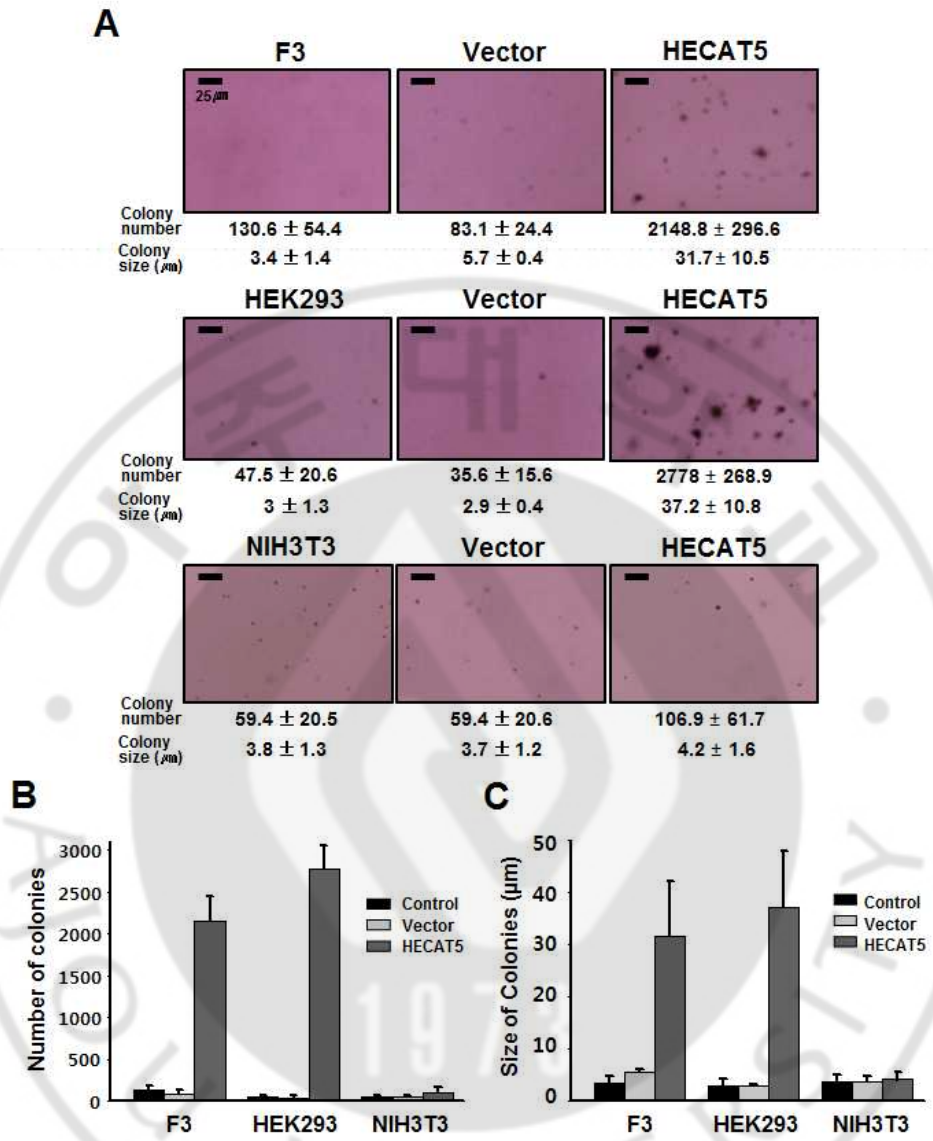
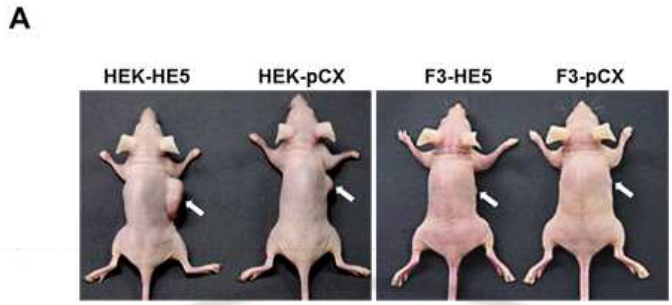


Fig. 2. Overexpression of HECAT5 increases tumorigenicity. (A) Soft agar assay in which cells were seeded at a density of 5×10^4 cells per $35mm^3$ dish and cultured in 0.35% soft agar in DMEM plus 10% FBS at 37°C for 4 weeks. The colonies were stained with 0.1% crystal violet. (B) Colony numbers in the entire dish were counted. (C) The average colony size was determined by measuring the diameters of colonies by a microcaliper.

C. Overexpression of HECAT5 promotes xenograft tumor formation.

To further investigate the potential effect of HECAT5 on tumorigenecity in vivo, F3, HEK293 cells with or without HECAT5 overexpression were subcutaneously inoculated into flank of athymic nude mice (F3 : n=6, HEK293 : n=8). After 11 days of inoculation, the mean volume of HECAT5 overexpressing tumors was significantly bigger than that of the control, which was observed in only HEK293 cell, not in F3 cell (Figure 3A and 3D). Two types of matrigel and cell number were used for identifying tumor formation of HECAT5 overexpressed F3 cell (Figure 3C). But, it didn't show any tumor formation in F3 cell. After a month, the mean weight of tumors with HECAT5 overexpression was considerably higher than that of the control (Figure 3E). Thus, these data suggest that HECAT5 may promotes xenograft tumor formation in vivo, in cell type-specific manner.



B

C

Cell No. injected (Matrigel Matrix type)		No. of mice with tumor/total mice			
		HB1.F3		HEK293	
		pCX	HECAT5	pCX	HECAT5
Growth factor reduced	1×10^6	0/3 (8wks)	0/6 (8wks)	1/8 (4wks)	8/8 (4wks)
	5×10^6	0/3 (8wks)	0/6 (8wks)	-	-
Regular type	1×10^6	0/3 (8wks)	0/3 (8wks)	6/6 (4wks)	6/6 (4wks)
	5×10^6	0/3 (8wks)	0/4 (8wks)	-	-

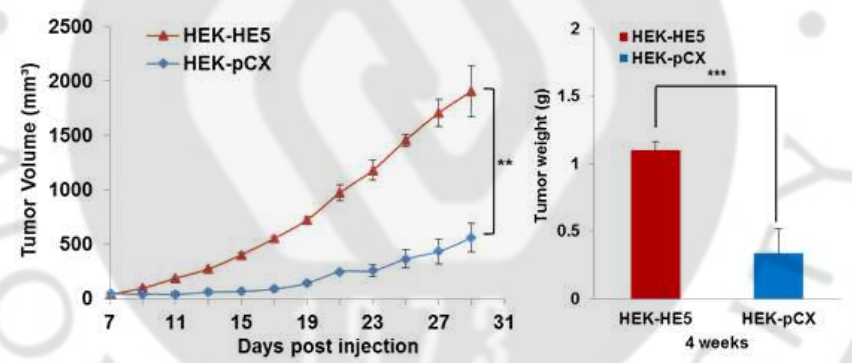


Fig. 3. Overexpression of HECAT5 promotes xenograft tumor formation. (A) HECAT5 overexpression increases xenograft tumor growth in only HEK293 cell. (B) Representative photograph of tumors developed in each group is shown. (C) Two types of matrigel and cell number were used for identifying tumor formation. (D) The average volume of tumors developed in each nude mice is shown as mean ± SE. (E) Tumors from each group were weighed directly after its removal. The average weight of tumors is indicated as mean ± SE.

D. mRNA and protein expression levels of the muscarinic acetylcholine receptor 3 in each cell lines

We examined the expression levels of muscarinic acetylcholine receptor 3 (M3R) in several different cell lines. A schematic representation of M3R is shown to detail the intron and exon structure and primers were designed. Reverse transcriptase-PCR (RT-PCR) was performed to determine the mRNA expression levels of M3R. GAPDH, employed as a positive control, expressed in all types of cells. M3R expressed in all types of cells, with the exception of NIH3T3. The M3R was expressed extremely high levels in HEK293 cell (Figure 4A). Western blotting was used to determine M3R protein expression in each cell lines, including F3, A4, HEK293, NIH3T3. Huh7 (Hepatocarcinoma cell), employed as a positive control. HEK293 cell demonstrated notably higher levels of phospho-M3R expression compared with another cell lines (Figure 4B).

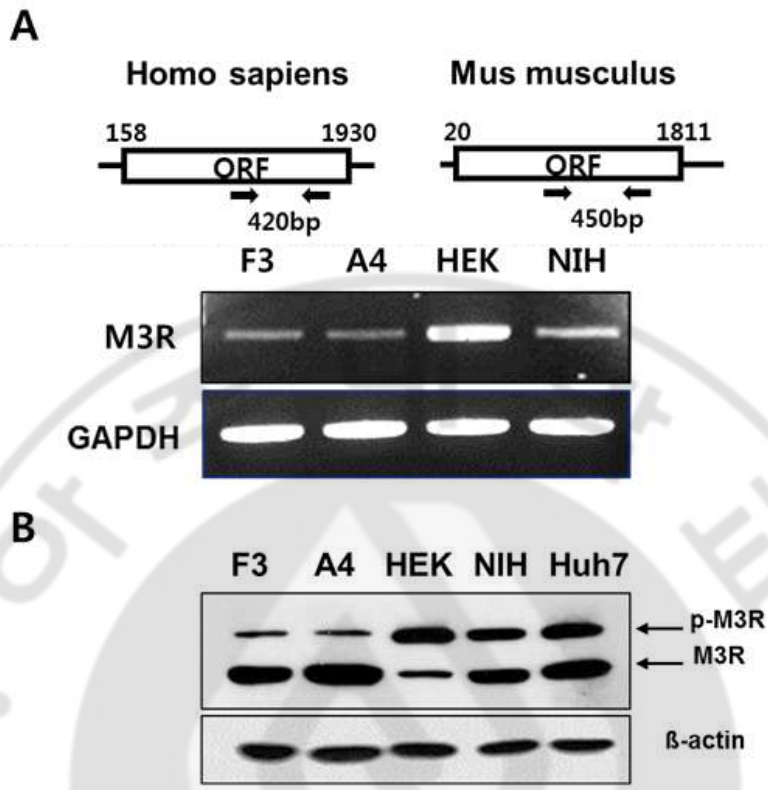


Fig. 4. Identification of a M3R expression in each cell lines. (A) Primer positions for Reverse transcriptase-PCR in human, mouse M3R exons. (B) human, mouse M3R mRNA expression levels were detected using reverse transcriptase-PCR, where GAPDH was used as an internal positive control. (C) human, mouse M3R protein expression levels were detected by western blot analysis, where β-actin was used as an internal positive control.

E. Regulation of cell proliferation by M3R modulators

Effects of M3R modulators on cell proliferation was monitored. Cells were seeded in 35mm^3 dishes at a density of 4×10^4 cells/dish, allowed to grow overnight. The cells were treated with carbachol and atropine (Figure 5B) for 72h at 37^{o}C. After the medium was removed, the cells were solubilized in 1% FBS in PBS and the cell number was measured using Countess automated cell counter (Thermo Fisher Scientific) and hemocytometer manual cell counting.

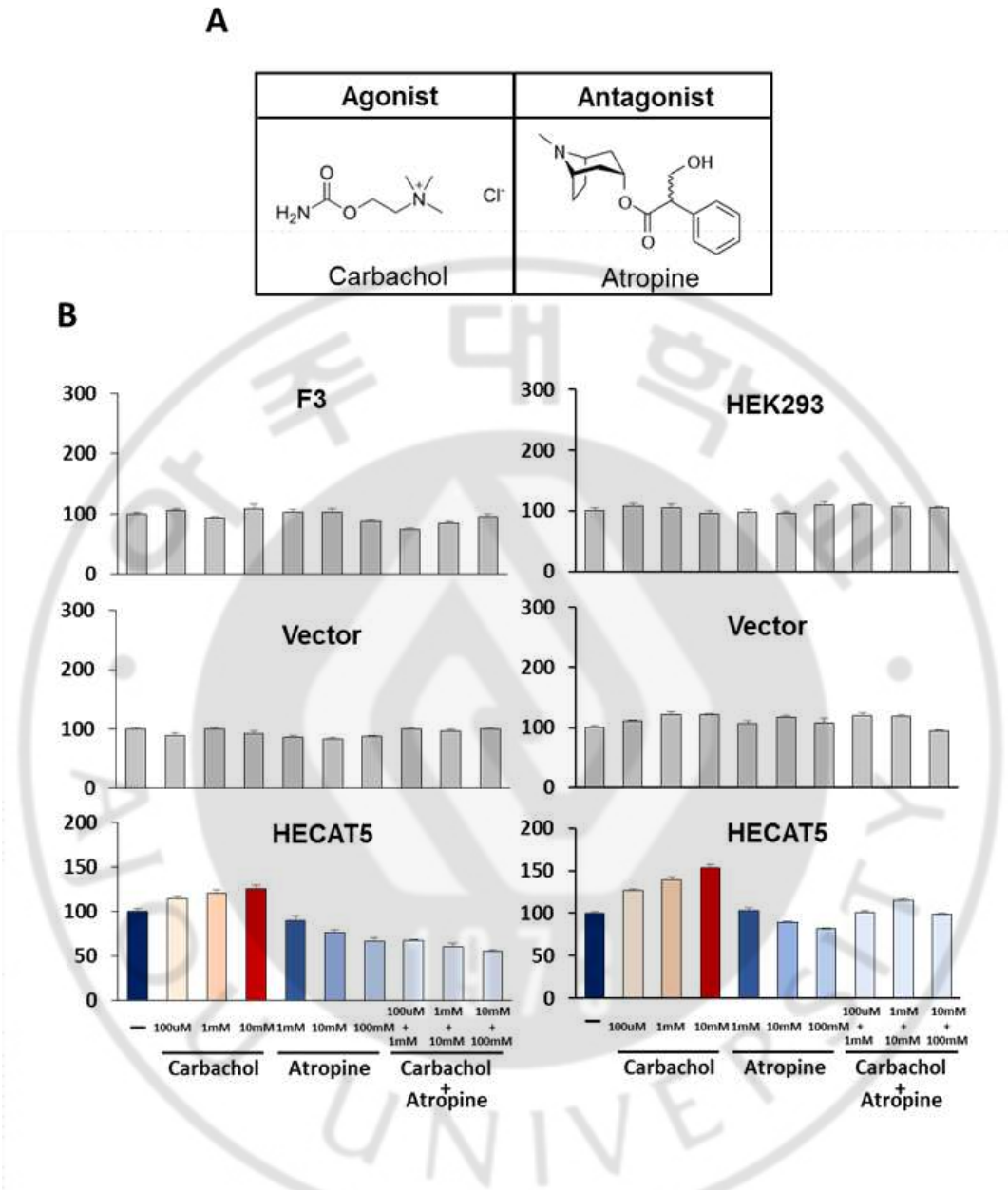


Fig. 5. Regulation of cell proliferaion by M3R modulators . (A) Chemical structure of the non-selective muscarinic agonist, carbachol and the antagonist, atropine. (B) Increasing concentrations of carbachol stimulate cell proliferation.

F. Cellular proliferation inhibited by M3R selective antagonists

Effects of M3R selective antagonists on cell proliferation was monitored. Cells were seeded in 35mm^3 dishes at a density of 4×10^4 cells/dish, allowed to grow overnight. The cells were treated with pfHHSiD and Darifenacin for 72h at 37^{o}C. After the medium was removed, the cells were solubilized in 1% FBS in PBS and the cell number was measured using Countess automated cell counter (Thermo Fisher Scientific) and hemocytometer manual cell counting.

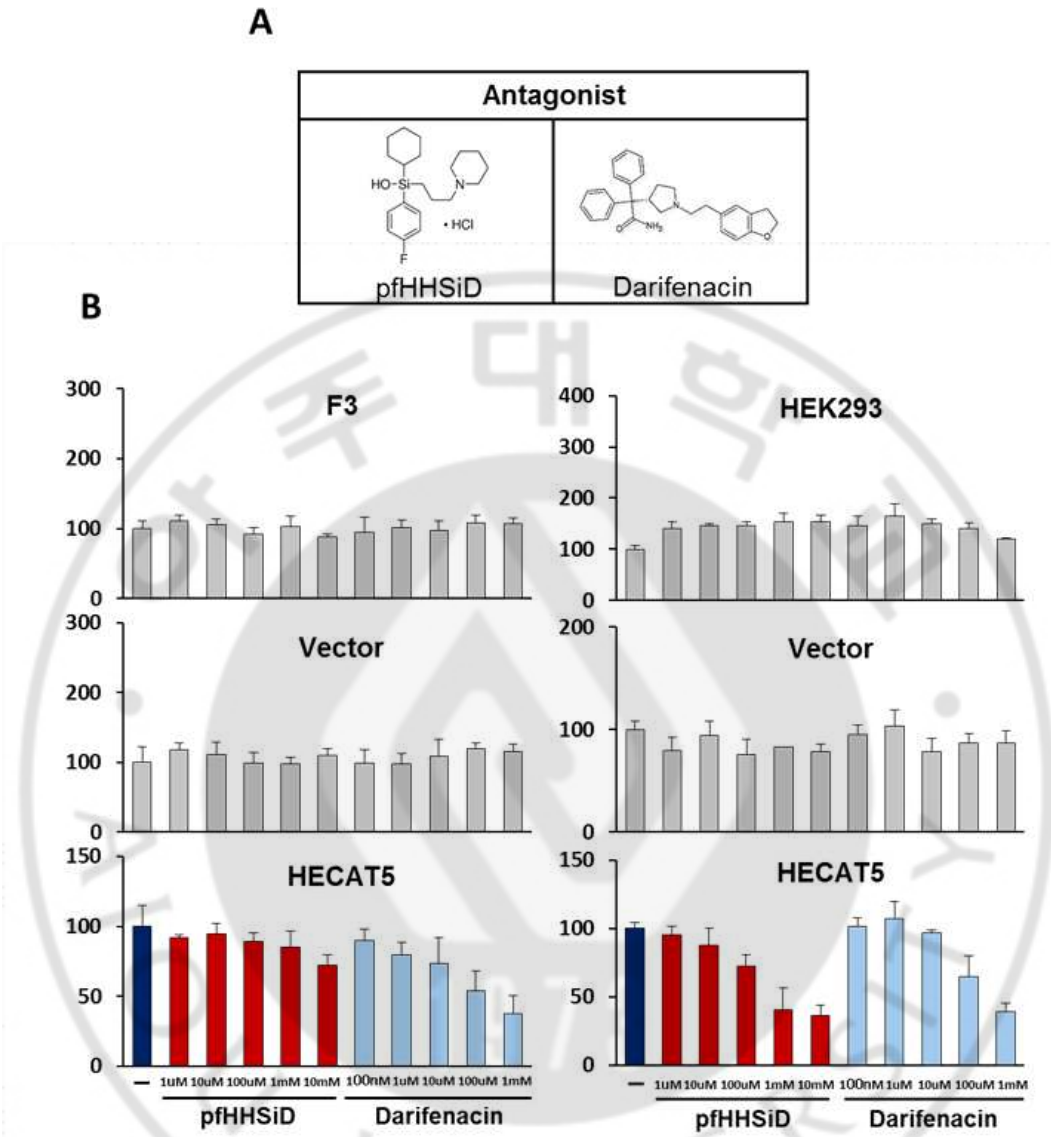


Fig. 6. Cellular proliferation inhibited by M3R selective antagonists. (A) Chemical structure of the selective muscarinic antagonists, pfHHSiD and Darifenacin. (B) Increasing concentrations of pfHHSiD and Darifenacin decrease cell proliferation.

G. Schematic indicating the phospho-acceptor sites in M3R and expression levels

In terms of the M3R, this receptor subtype can be phosphorylated by multiple phospho-acceptor sites. This revealed 14 serine, 1 threonine and 1 tyrosine phospho-acceptor sites in the third intracellular loop and one serine in the C-terminal tail (Figure 7A).

To detect protein expression level of M3R in three cell lines, Western blot assay were used. In Western blot assay the expression pattern of M3R was significantly different between F3, NIH3T3 and HEK293 cells. In F3 and NIH3T3 cells, native expression level of M3R was higher than phospho-M3R. Conversely, HEK293 cells showed considerably higher levels of phospho-M3R than native M3R. These results indicate that levels of M3R transcription and translation were dissimilar in cell lines (Figure 7B).

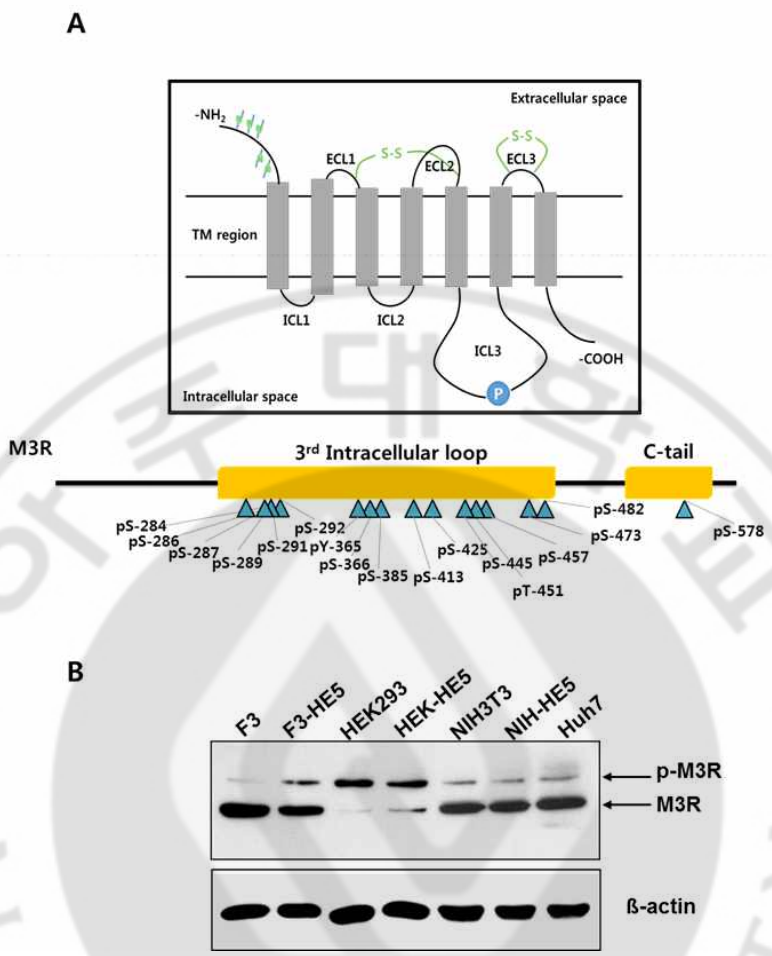


Fig. 7. Schematic indicating the phospho-acceptor sites in M3R and expression levels. (A) The structure of human M3R (B) M3R protein expression levels were identified by western blot. p-M3R is phophorylation levels of M3R. In F3 cells, phosphorylation level of M3R was relatively low but HECAT5 enhanced its level. Meanwhile, in HEK293 cells, basically its level was high but HECAT5 decreased it.

H. Differential expression of ERK and AKT pathways in three cell lines and principle of GPCR signaling

The ERK and Akt signaling cascade plays a important role in various physiological processes, including cell cycle progression, transcription, apoptosis, and metabolism. Especially, the ERK and Akt pathway is known to regulate the function of numerous substrates, which regulate cellular growth and cell cycle progression in GPCR pathway. Collectively, our results suggest that the ERK and Akt signaling pathway play a important role in active cell proliferation of F3, HEK293 cells, respectively (Figure 8A).

G protein-coupled receptors are the largest family of transmembrane receptors and are responsible for the transduction of a diverse extracellular signals. When a ligand binds to the GPCR it causes a conformational change in the GPCR. The GPCR can then activate an associated G-protein by exchanging its bound GDP for a GTP. There are two principal signal transduction pathways involving the GPCRs : the cAMP and PIP_2 signaling pathway. In a current model, phosphorylated receptors recruit β-arrestins which are adaptor proteins that block further heterotrimeric G protein coupling (desensitization). β-arrestin also mediate independent signaling pathway downstream of GPCRs, ERK. These two signaling states of the receptor are mutually exclusive.

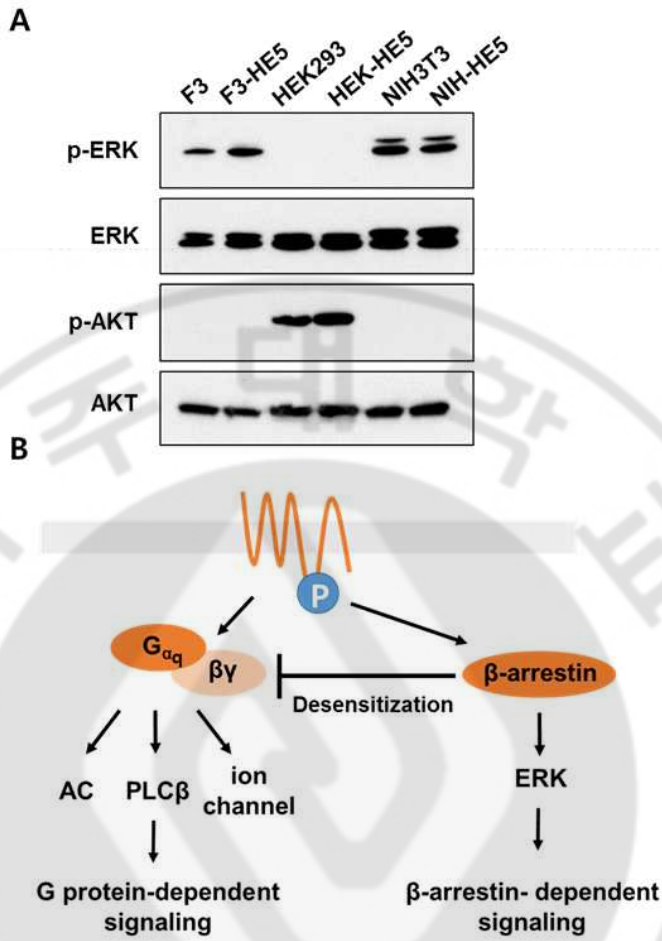


Fig. 8. Differential expression of ERK and AKT pathways in three cell lines and principle of GPCR signaling. (A) Western blot showed the differential expression levels of p-ERK, p-AKT in cell type-specific manners. Total protein levels of ERK, AKT were used as respective internal controls. (B) Activated receptors couple to heterotrimeric G proteins. Subsequently, it dissociate and G protein signaling mediates the generation of second messengers (G protein-dependent signaling). Activated receptors are phosphorylated recruit β-arrestins block G protein-GPCR coupling and scaffold ERK (β-arrestin-dependent signaling).

I. A different signaling pathways of M3R-HECAT5 in cell type-specific manner

GPCRs (G-protein coupled receptor) do not activate all cellular signaling pathways in a uniform manner. GPCRs can differentially activate G-proteins or β-arrestins. In case of GPCR phosphorylation, it recruits β-arrestin which function to terminate or inhibit canonical G-protein signaling and initiate distinct β-arrestin-mediated signaling pathways. GPCR phosphorylation is a complex process involving a multiple different protein kinases able to phosphorylate the same receptor at different sites. This site-specific phosphorylation would result in a specific signaling outcome. In this way, receptor phosphorylation might act as a dynamic and flexible regulatory process in a cell type-specific manner.

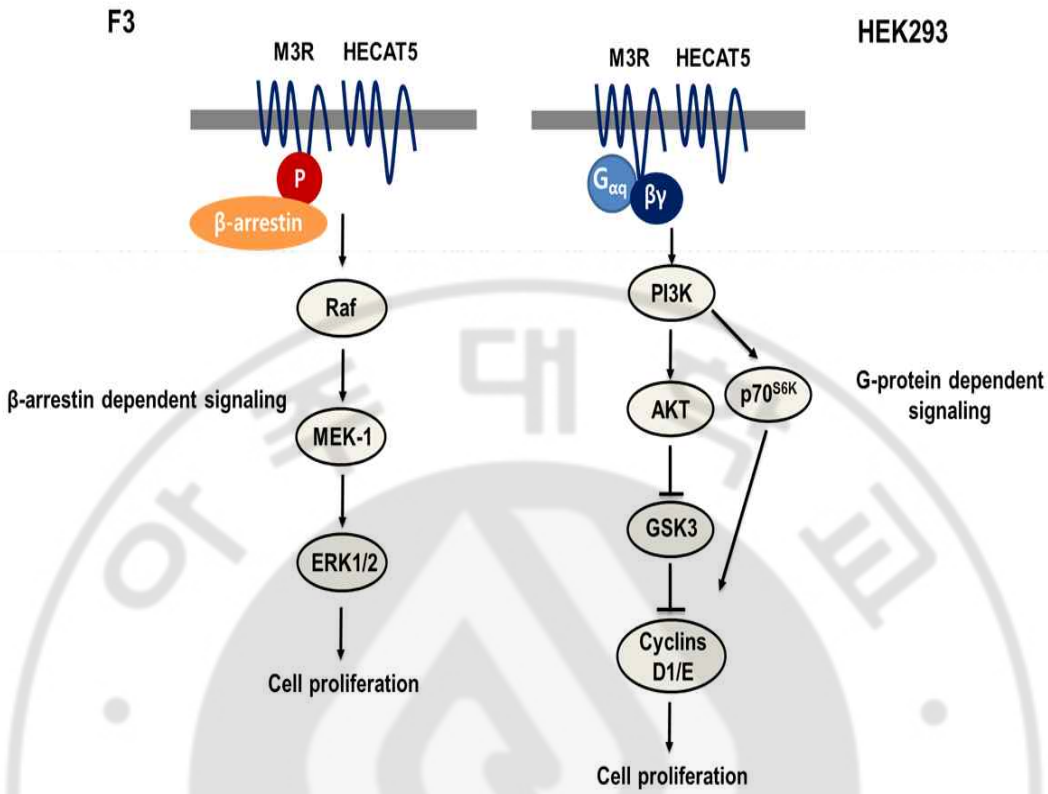


Fig. 9. A different signaling pathways of M3R-HECAT5 in cell type-specific manner. (A) Site-specific M3R phosphorylation of F3-HECAT5 results in the switching off of G-protein signaling, while, at the same time, inducing Raf-dependent ERK1/2 activation. (B) Activation of PI3K-dependent cell cycle regulation in HEK293 cell. PI3Ks may also promote proliferation by promoting cyclin expression (via p70S6K).

Ⅳ. DISCUSSION

In recent years there is a tremendous expansion of research focused on the embryonic stem cell biology (Hadjimichael C et al, 2015). One of the most important issues in embryonic stem cell biology is understanding the mechanisms that regulate self-renewal. Self-renewal is crucial to ES cell function, because it is required by many types of stem cells to maintain for its whole life. Moreover, whereas stem cells from different organs may vary in their developmental potential, all stem cells should self-renew and regulate the relative balance between self-renewal and differentiation (Tannishtha Reya et al, 2001).

hESCs can be derived from fetal tissue after abortion (Bernard Lo et al, 2009). However, there are problems for hESC use in cell replacement therapy. One is the immune incompatibility problem between the donor cells and the recipient cells. It can result in the rejection of transplanted cells. The other is ethical problem, because the embryo dies during the isolation of ESCs (S.P. Medvedev et al, 2010). In 2006, a new type of pluripotent cells was obtained, induced pluripotent stem cell (iPSC). Reprogramming somatic cells to iPS cells by defined 4 transcription factors may provide vast opportunities for developing regenerative medicine (Jiarui Wu et al, 2014). However, there are some problems of iPSC production efficiency and application safety in cell replacement therapy.

To investigate the mechanism regulating stemness, especially self-renewal, we identified a hESC-specific HECAT5 by Digital differential display (DDD). Functionally, HECAT5 regulates stemness characteristics, such as self-renewal, tumorigenesis, through the interaction with M3R. We also found that HECAT5 endows these properties through the

different signaling pathway. This is the first discovery to reveal that HECAT5 regulates stemness. Although multiple molecules and signaling pathways have been shown to associate with the stemness, the regulatory mechanisms of HECAT5 are not yet completely elucidated. Ultimately, we wish that HECAT5 can be used as a substituents for c-MYC in iPS-inducing.

Ⅴ. CONCLUSION

In conclusion, we demonstrated that hESC-specific HECAT5 possesss stemness properties. HECAT5 enhances self-renewal ability in F3 and HEK293 cells. The interaction of HECAT5 with M3R is important for HECAT5's function. In addition, HECAT5 interacts with ERK and AKT in cell type-specific manner. What remains to be a question is the exact regulatory mechanism of HECAT5 expression in each cell lines. After this confirmation, we wish that it can be used as a potent substituents for c-MYC in iPS-inducing.

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-국문요약-

세포유형에 따른 HECAT5와 M3R의 상호작용을 통한 세포증식 조절

아주대학교 대학원 의생명과학과

우 창 성

(지도교수: 이 명 애)

배아줄기세포는 초기 포유류 배아의 만능 내세포 덩어리에서 얻어진다. 배아줄기세포의 자기재생과 다분화능은 줄기세포 치료를 위해 매력적인 물질이다. 그러나 윤리적이고 실용적인 이유 때문에 배아줄기세포는 잠재적 치료 응용 프로그램에 제한이 있다. 특히 배아줄기세포의 세포주기 관련 줄기세포능을 조절하는 기전을 조사하고자 우리는 이전연구에서 DDD를 통해 배아줄기세포와 암세포에서 공통으로 발현하고 정상조직에서는 발현하지 않는 유전자, HEACT5를 발굴하게 되었다.

HECAT5를 3종류의 세포에 과발현시킨 후 확인해보니 세포 유형에 따라 자기재생과 종양형성 능력을 촉진시키는 것을 확인하였다. HECAT5는 상호작용 파트너로 알려진 후보물질 M3R과 결합을 통해 이러한 영향을 보이고 있었다. 이는 M3R 활성의 조절물질인 agonist와 antagonist를 통해 HECAT5가 과발현된 세포에서만 반응을 보이는 것을 확인함으로써 증명하였다. 게다가, HECAT5는 각 세포유형에서 서로 다른 수준의 M3R 인산화정도를 보였다. F3 세포에서 M3R의 인산화정도는 상대적으로 낮았는데 HECAT5가 과발현된 F3 세포에서 그 발현양이 증가하였다. 반면에, HEK293 세포에서는 기본적으로 인산화된 M3R

수준이 높았지만 HECAT5의 과발현에 의해 그 정도가 약간 감소하였다. 이외에도 F3 와 HEK293 cell은 각각 ERK와 AKT에만 반응하는 서로 다른 신호경로를 보였다. 종합하여 보자면, 우리가 새롭게 발굴한 인간배아줄기세포 특이적 HECAT5는 세포 유형별로 서로 다른 반응 경로를 통해 줄기세포능을 조절함을 알 수 있었다.

핵심어 : 배아줄기세포, 자기재생, 다능성, 세포증식, 줄기세포능