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The role of SNX26 in EGFR-mediated circular dorsal ruffle formation

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논문 제목: **The role of SNX26 in EGFR-mediated circular dorsal ruffle formation**

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ABSTRACT

The role of SNX26 in EGFR-mediated circular dorsal ruffle formation

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A large number of plasma membrane structures induced dynamic changes by a variety of mechanical and chemical stimuli. Circular dorsal ruffles (CDRs) are dynamic and transient actin-rich ring-like membrane structures that arise from the dorsal side of mammalian cells treated with various growth factors. Physiological functions of CDRs are implicated in the down-regulation of growth factor receptors and the migration of cells. However, the precise mechanism by which the dynamics and formation of CDRs are regulated is largely unknown.

Here, we show that Sorting nexin 26 (SNX26), a brain-enriched Rho GAP containing protein with multiple signaling domains, interacts with actin and

regulates the formation of CDRs via the Rho GAP domain in COS-7 cells. SNX26 interacts with actin via its PX and Rho GAP domains, but not SH3 and PRD domains. Overexpression of SNX26 full-length and its RhoGAP domain-containing deletion mutants decreased the filamentous actin content in COS-7 cells by ~50%. Overexpression of N-terminal comprising of PX, SH3 and Rho GAP domains inhibited CDR formation induced by epidermal growth factor (EGF) in cells. On the other hand, N-term R350I, which is the GAP defective mutant form of N-term, increased the number of cells forming CDRs in response to EGF. Interestingly, although PX and PX Y100A (defective mutant of phospholipid binding affinity) domains are capable of binding to actin, they cannot affect the formation of CDR. Moreover SNX26 has the capability for intra- or inter-molecular interaction through PX and Rho GAP domains.

These results indicate that SNX 26, acting as a Rho GAP, regulates actin cytoskeleton and modulates CDR formation by EGF treatment in cells. Furthermore its function might be regulated by intra/inter-molecular interaction event. Taken together, it has raised possibility that SNX 26, acting as a regulator of cytoskeletal remodeling, has a key role in EGFR-mediated CDR formation via Rho protein signaling pathway in cells.

Keywords: circular dorsal ruffle, EGFR(epidermal growth factor receptor) macropinocytosis, SNX(sorting nexin family) 26

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CONTENTS

LIST OF FIGURES

Ⅰ. Introduction

Circular dorsal ruffles or waves (CDRs, also known as actin ribbons or dorsal ruffles) are distinct actin-based membrane apparatus mostly found on the dorsal side of plasma membrane treated with a variety of receptor-tyrosine-kinase (RTK) growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and hepatocyte growth factor (HGF) in various cell types [1, 2]. RTKs have important functions in biological processes as regulators of cell differentiation, proliferation, migration, innate immune response and angiogenesis [3]. However, the detailed molecular mechanisms of how these RTKs contribute to such actin structures still remain unclear. As a matter of fact, many membrane surface structures- podosome, invadopodia and linear ruffles- with CDRs less amply distinguished in content and function up to this time. Podosomes predominantly comprise of dot-form (1~2μm diameter) and localized adjacent to plasma membrane [4]. Invadopodia is accompanied by degradation of the extracellular matrix and are situated at perinuclear compartments in cancer cells [5, 6]. Peripheral ruffles established within a minute after stimulation with growth factors and immediately ceased [3].

On the other hand, CDR has been discovered a few characters in shape, life-time and location. After stimulation with growth factors, these ruffles

assembled within a few minutes and disappeared rapidly (5-20min). Plus, it has largest diameter among those membrane structures. It has been worked that during CDRs occur in cells , not only the activators of actin polymerization, such as N-WASP and the Arp2/3 complex, but also membrane deforming proteins are necessary [2]. Although CDR has been long observed in cells, the physiological function is still enigmatic. Previous studies have given a brief account of these dynamic structures that CDR is associated with macropinocytosis as well as cell migration by providing rearrangement of the actin cytoskeleton [7]. Macropinocytosis, a sort of fluid-phase endocytosis, is applied a wide range of actin reorganizations in advance of cell motility and growth factor receptor internalization. This pathway is not independent endocytic coat proteins including clathrin and caveolin [8, 9]. A recent study provided through the experiment that expressing GFP tagged EGFR existed and internalized via circular dorsal ruffles, but it had not occur in clathrin and caveolin-dependent endocytic pathways. It indicates that aspect of macropinocytosis pathway is similar to CDR [10, 11].

Cdc42, Rac1 and RhoA are small GTP-binding proteins of the Rho family, a subfamily of the Ras superfamily. It is known that Rho proteins, acting as molecular switches, are responsible for governing the formation of cellular protrusions [12]. They perform cycling between an active GTP-bound form and an inactive GDP-bound form. This activity is modulated by a combination of three classes of proteins: GTPase-activating proteins (GAPs), guanine nucleotide dissociation inhibitors (GDIs), and guanine nucleotide exchange

factors (GEFs). GAPs stimulate an otherwise low intrinsic GTPase activity, reverting the conformation back to the inactive GDP-bound form. GDI maintain small GTPases in the inactive state but remove these GTPase by cytosolic factor. GEFs are in response to activated GTPases which interact with one or more effector proteins, leading to activation of downstream signaling pathways [13]. These regulators have been reported that involved in the processes like macropinocytosis or ligand induced internalization of RTK. Various studied shown that both RhoA and Cdc42 have been implicated in the regulation of actin-rich structures in osteoclasts and HeLa cells [14].

The sorting nexins (SNXs) are a diverse group of proteins that characterized potential importance of membrane structures through their lipid-binding PX domain (a phospholipid-binding motif) such as membrane trafficking, cell signaling, membrane remodeling and organelle motility [15] . Some members of this family have been implicated in macropinosome biogenesis in cultured cells. The SNX-PX-BAR family was discovered that promote macropinosome formation on macropinocytosis [16]. Especially, SNX 5 interacts with Phosphatidylinositol 3-phosphate (PI3P) and Phosphatidylinositol (3, 4)-bisphosphate ($PI(3,4)P_2$) via its PX domain and regulates macropinocytosis as well as macropinosome maturation [17, 18].

Here, we studied about SNX26. There are other nomenclature; ARHGAP33, TCGAP, NOMA-GAP. (Rho GTPase activating protein 33, TC10β and Cdc42 GTPase-activating protein, Neurite outgrowth multi adaptor-GAP) It consists of Phox homology (PX) domain, Src homology 3 (SH3) domain, a

Rho GAP domain and repeated proline-rich domain (PRD) at the C-terminus. It is shown that SNX26 mRNA highly expressed in brain and testis. Functions of SNX26 has been mainly reported dendritic arborization and cortical thickness via Rho GAP activity in neuron, although it has been studied the regulator as glucose transporter 4 in response to insulin stimulation in adipocytes [19][20].

In the present study, we have analyzed the number of CDR in response to EGF stimulation in COS-7 cells [10]. We describe a function of SNX26 through its Rho GAP domain, but not PX domain, as a regulator of cytoskeletal remodeling and its PX domain. Then, the Rho GAP domain raises the potentiality that Rho pathway is a target for EGFR mediated signaling in fibroblast cell.

Ⅱ. Materials and Methods

1. Cell culture and transfection

The monkey kidney cell line COS-7 cells (Korean Cell Line Bank, Seoul, South Korea) and Human embryo kidney cell line HEK293T cells (ATCC, Manassas, VA, USA) were maintained in DMEM(Invitrogen, San Diego, CA, USA) supplemented with 10% FBS (Hyclone, Logan, UT, USA) at 37℃ in 5% CO_2.Plasmid transfection was performed using Lipofectamine 2000 reagent (Invitrogen) . Experiments were carried out 48 hr after transfection.

2. Plasmids, antibodies and reagents

SNX26 constructs originated from human was kindly provided by Dr. Tadashi Yamamoto (University of Tokyo, Tokyo, Japan). We used antibodies GFP (Abcam, Cambridge, UK) and anti-HA (Covance, Princeton, NJ). HRP conjugated secondary antibodies were obtained from Jackson Immuno Research Laboratories (West Grove, PA). Fibronectin was from Human plasma (Sigma-Aldrich). Human Recombinant Epidermal growth factor (EGF) was purchased from Milipore (Temecula,CA) .

3. Immunoprecipitation and Western blotting

Lysates of transfected HEK293T cells , to investigate which domains of SNX26 interaction with Actin, were prepared using lysis buffer (10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1mM sodium orthovanadate, 1 mM PMSF, 10 mM leupeptin, 1.5 mM pepstatin, and 1 mM aprotinin) for 1 hour at 4°C. To clarified the lysates by centrifugation at 15,000 g for 15 min at 4°C, and incubation for 4 hours at 4°C with anti-GFP antibody, and then incubation again for binding with protein A-Sepharose beads (Amersham Biosciences) for 2 hours at 4°C . The Immunoprecipitate and lysates were resolved by SDS-PAGE and transferred onto polyvinylidenendifluoride (PVDF) membranes (Bio-Rad). The membrane was blocked with 5% skim milk in TBS-T for 90min, washed, and probed with primary antibody for 2 hours at RT. the membrane was washed with TBS-T 3times every 5min, incubated with HRP-conjugated secondary antibody (Jackson Immuno Research Laboratories). Proteins were visualized with ECL reagent (Amersham Biosciences)

4. Immunofluorescence microscopy

To visualization SNX26 and F-actin, co-transfected cells were washed with Tyrode's solution, and then fixed in 4% paraformaldehyde(PFA), 4% sucrose, phosphate-buffered saline (PBS) for 15 min, washed, and permeabilized for 5 min in 0.25% Triton X-100, washed. To blocked the cells, incubated for 30 min in 10% bovine serum albumin (BSA), PBS at 37℃ and incubated with

Texas Red-phalloidin in 3% BSA, PBS for 30 min at 37℃.

Images were acquired with a Zeiss Axiovert200M inverted microscope (Carl Zeiss, Oberkochen, Germany) installed with a 40x oil-immersion objective lens, N.A. 1.0 using an ORCA-R2 CCD camera (Hamamatsu Photonics, Hamamatsu, Japan). We used imaging software 'MetaMorph' (Molecular Devices, Sunnyvale, CA) with a GFP- or amRFP-optimized filter set (Omega Optical, Brattleboro, VT).

5. Assay of circular dorsal ruffle formation

COS-7 cells were maintained in DMEM with 10% FBS on fibronectin-coated (10μg/ml) coverslip. The effects of the SNX26 fragments in response to epidermal growth factor (EGF). The cells were transfected with the plasmids directing expression of GFP-tagged SNX26 domain using Lipofectamin 2000 (Invitrogen). 24 hr after transfection, the cells were incubated low-serum DMEM with 0.2% FBS for 18 hr, followed by washing 3times with PBS and then treatment with 400 ng/ml EGF for 5 min on 37℃ heat plate. Then, the cells were fixed with 4% PFA for 15 min at RT, permeablized with 0.1% TritonX-100 for 2 min. To visualized actin structures immunostained with Texas -Red phallodin.

6. Quantification of the number of CDR and F-actin contents

Analysis and quantification of data were worked with MetaMorph software program. To count positive-CDR cells, the intensity is higher than background

signal (>30) was indicative of GFP-positive transfected cell. And It has nothing to do with the size and number of CDR within one cell.

F-actin statistical analyses were carried out with PASW Statistics 18 (IBM, Armonk, NY). Data showed as means ± S.E. For multiple conditions, we compared means by analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) *post hoc* test to clarify statistically different groups.

Ⅲ. Results

The Rho GAP domain of SNX26 is responsible for reduction of F-actin content.

It has been known that SNX26 is highly expressed in brain and testis. Previous studies reported that SNX26 regulates early neuronal development as well as dendrite branching in neurons. To investigate actin cytoskeleton reorganization properties of SNX26 as Cdc42 specific GTPase activating protein (GAP) in non-neuronal cells, the green fluorescent protein (GFP) tagged each domain of SNX26 was transfected for 24 hr into COS-7 cells, and then the cells were immunostained with Texas-Red conjugated phalloidin for filamentous actin (F-actin) stain. As shown in Figure 1A, full-length of GFP-SNX26 was expressed in cells and F-actin content markedly decreased compared with that in GFP transfected control cells. Similarly, cells expressing GFP-delta PRD and Rho GAP domains showed over 50 percent predominant reduction of F-actin content compared with that in cells expressing GFP alone (Figure 1C). These data suggest that the GAP activity alone causes a change in actin cytoskeleton reorganization in COS-7 cells.

PX and Rho GAP domains interact with actin.

We observed F-actin content was impacted on SNX26 fragments via immunostaining. To test whether SNX26 and any deleted mutant of SNX26 interacts with actin, each GFP-tagged SNX26 full-length or deleted mutant (PX, SH3, Rho GAP and PRD) constructs was co-transfected with HA-actin in HEK293T cells for 48 hr. It was subjected to immunoprecipitation with anti-GFP antibody and immunoblotting with anti-HA antibody. Even though Rho GAP domain expression levels were low, binding of the wild type PX domain and Rho GAP domain to actin was well-observed (Figure 2B). Preceding studies have suggested that PX domain is known for participation in membrane attachment to organelles of the secretory and endocytic system via binding of phosphoinositide lipids. Especially, it was also reported that PX domain of SNX26 interacts with PtdIns(3,4)P_2, PtdIns(4,5)P_2 and PtdIns(3)P. In order to explore the important of this domain in SNX26, we generated the mutated constructs that reduce binding affinity to phospholipid. These are the consensus tyrosine was mutated to alanine in PX domain and N-terminal fragment of SNX26 (PX Y100A/N-terminal PX Y100A). Plus, we made two mutated constructs that failed as a GAP (Arg-350 to Ile-350; R350I), the one is N-terminal Rho GAP R350I and the other is Rho GAP R350I. We then investigated whether PX Y100A and Rho GAP R350I domains interact with actin by immunoprecipitation assay. As a result, all these mutants have increased binding affinities to actin compared with wild type (Figure 2C). Taken together, we suggested in these results that both of PX and Rho GAP

mutation regions are important to interaction with actin and this point may attenuates actin simultaneously an event.

PX domain of SNX26 had no effect on circular dorsal ruffle formation.

Circular dorsal ruffles or waves are respond to stimulation by a variety of receptor-tyrosine-kinase growth factors, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and hepatocyte growth factor (HGF), then established ring-like shape actin structures through actin cytoskeleton reorganization. Recent work, some SNX families-SNX1, SNX5, SNX9, SNX18 and SNX33- were all in promotion of macropinosome formation and association with early-stage macropinosome. Recent work had the aspect that CDR formation is as a mediator of macropinocytosis, we asked whether SNX26 influence CDR formation via PX and Rho GAP domains, which interacted with F-actin. First of all, we tested PX domain modulates actin. COS-7 cells were transfected with GFP tagged wild type PX and PX Y100A for 24 hr and then the cells were starved for 18 hr and treated 400ng/ml EGF for 5 min. The cells were fixed and followed by immunostaining with Texas Red-phalloidin to detect F-actin. As shown in figure 3A, PX Y100A less localized at the plasma membrane compared with wild-type PX domain. We analyzed the number of CDRs in transfected cells. Wild-type PX and PX Y100A slightly increased or decrease the percentage of CDRs as compared with control, but both of them did not have any effect on

CDR formation. Therefore, both wild-type PX and PX Y100A did not have any effect on CDR formation.

Rho GAP R350I has a critical role of CDR formation

We investigated the effect of Rho GAP domain on the number of CDR formation that is another domain to interact with actin. The wild-type N-terminal fragment, which contains PX, SH3 and Rho GAP domains, was found in the nucleus distinctively and seemed to shrink at cytoplasmic region. Also, it seems to be the result of defects in CDR formation. On the other hand, Rho GAP-induced decrease in the number of cells containing CDRs was efficiently rescued by Rho GAP R350I, a GAP defective Rho GAP, indicating that the GAP activity is responsible for its effect on CDR formation. To examine whether a change for the number of cells containing CDRs between wild type N-term and containing PX Y100A mutant of N-terminal fragment in same physiological condition. In the statistics, control had higher CDR in compared to wild type N-term by approximately 3.9 fold. Likewise, N-terminal Rho GAP R350I increased the number of CDRs by 2.6 fold compared with those formed in cells expressing wild type N-terminal. Even though N-terminal PX Y100A increased slightly versus wild type, it was not significant tendency (Figure 4B). Further, supporting the hypothesis that Rho GAP activity is necessary for precise control of CDR formation.

intra/inter-molecular interaction of SNX26

Although both PX and Rho GAP domains bind to actin, they had different appearance in CDR formation. The number of cells containing CDRs was slightly increased in cells expressing PX domain, whereas it decreased the number in cells expressing Rho GAP domain. Also, overexpression of full-length SNX26 was responsible for aggregation in cells (Figure 1A). HEK293T cells were co-transfected with HA- tagged full-length SNX26 and GFP-tagged SNX26 (full-length, PX, SH3, Rho GAP and PRD) constructs for 48 hr and the cells were subsequently immunoprecipitated with GFP antibody. We observed the binding of PX domain to Rho GAP domain. We then performed immunoprecipitation and immunoblotting to examine binding affinity between PX Y100A and Rho GAP domain. As a result, there was no distinction with wild-type PX domain (data not shown). Ironically, the test with PRD domain, Even though it interacts with full-length SNX26, there was no interaction between PRD domain and other domains. Potentially, the PRD domain is in a state of skewed conformation by oneself.

Here we explored some relationships not only actin cytoskeleton, but also self-association of SNX26 further supporting the hypothesis that SNX26 has several functions of activator about actin as well as negative regulator itself.

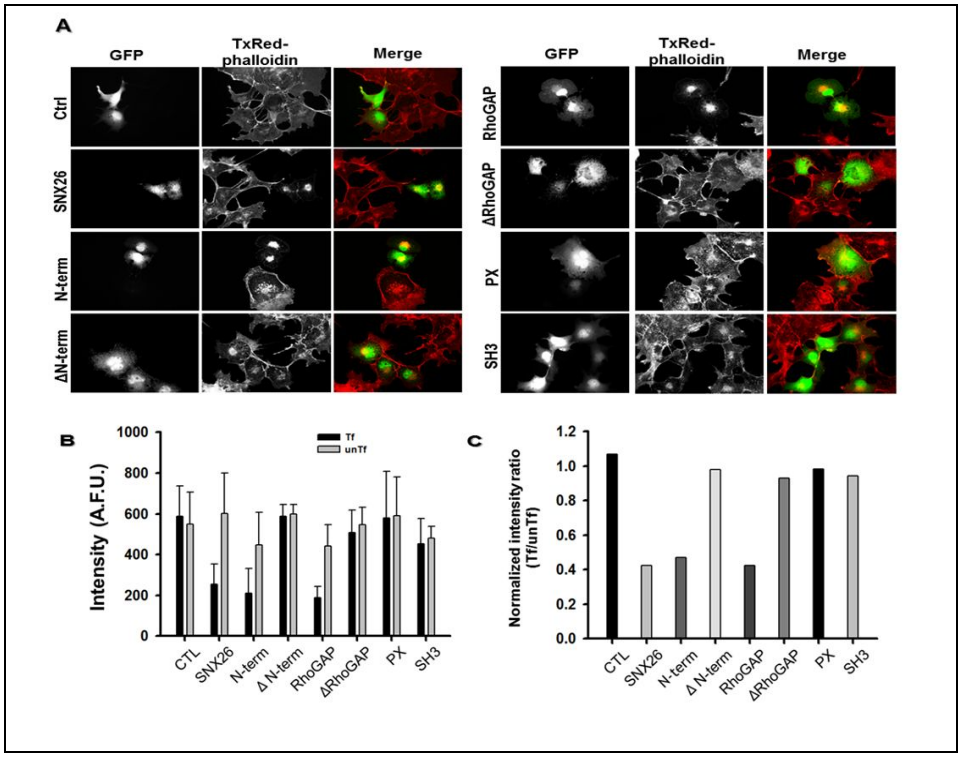

Figure 1. Rho GAP domain of SNX26 reduces cytosolic F-actin content.

(**A**) Representative images for SNX26-induced modulation of F-actin contents. COS-7 cells expressing GFP or GFP-tagged indicated mutants of SNX26 were fixed and stained with Texas Red-phalloidin for visualizing F-actin contents. Scale bar, 10 ㎛. (**B**) Arbitrary fluorescence unit (A.F.U.) of Texas Red intensity in each group was measured. >40 cells were analyzed for each group. Data are presented as means ± SE of three independent experiments. Tf stands for transfected cells and unTf stands for adjacent untransfected cells. (**C**) In each group, Texas Red intensity of Tf was normalized to that of unTf. Snx26 containing the Rho GAP domain decreases in F-actin contents in cells.

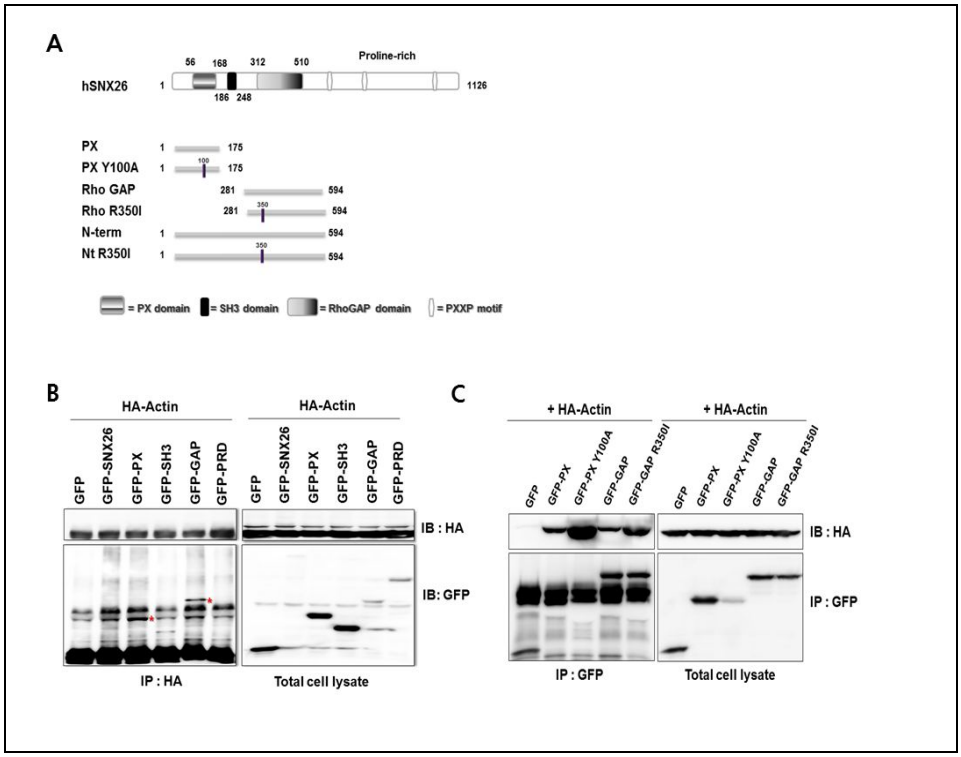

Figure 2. PX and Rho GAP domain interaction with F-actin

(A) Schematic diagram of human SNX26 and point mutants used in this study (**B, C**) both of PX and Rho GAP domain bind to actin. HEK293T cells were co-transfected GFP vector control or GFP- tagged full length of SNX26, PX, SH3, GAP, PRD and different mutants of SNX26 (C;PX Y100A, GAP R350I) in a combination with HA-tagged Actin for 48 hr, co-immunoprecipitation (IP) was carried out cell lysates and agarose beads conjugated with anti-HA (B) or anti-GFP-antibody (C). Membranes were immunoblotted with HA and GFP-antibodies.

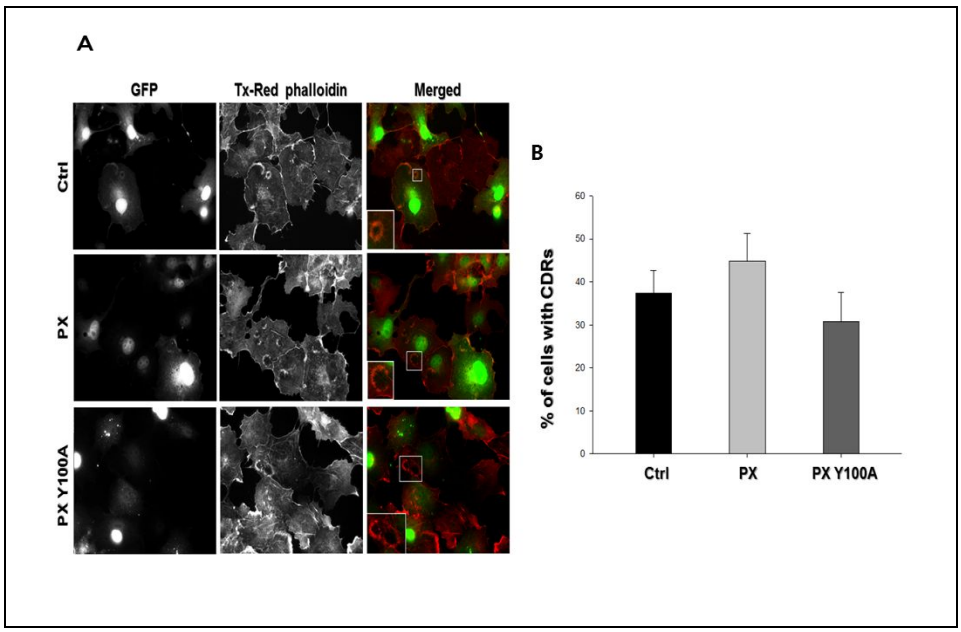

Figure 3. PX domain of SNX26 had no effect on circular dorsal ring formation

(**A**) COS-7 cells grown on fibronectin-coated(10μg/ml) coverslip and expressing GFP vector control or GFP-tagged PX, PX Y100A were transfected for 24 hr and then starved for 18 hr and stimulated with 400 ng/ml EGF for 5 min at 37℃ and fixed and permeabilized. To visualize actin-filaments, the cells were also stained Texas Red-phalloidin. Insert show CDR structures. (**B**) Presented is the percentage of the cells have CDR within transfected cells

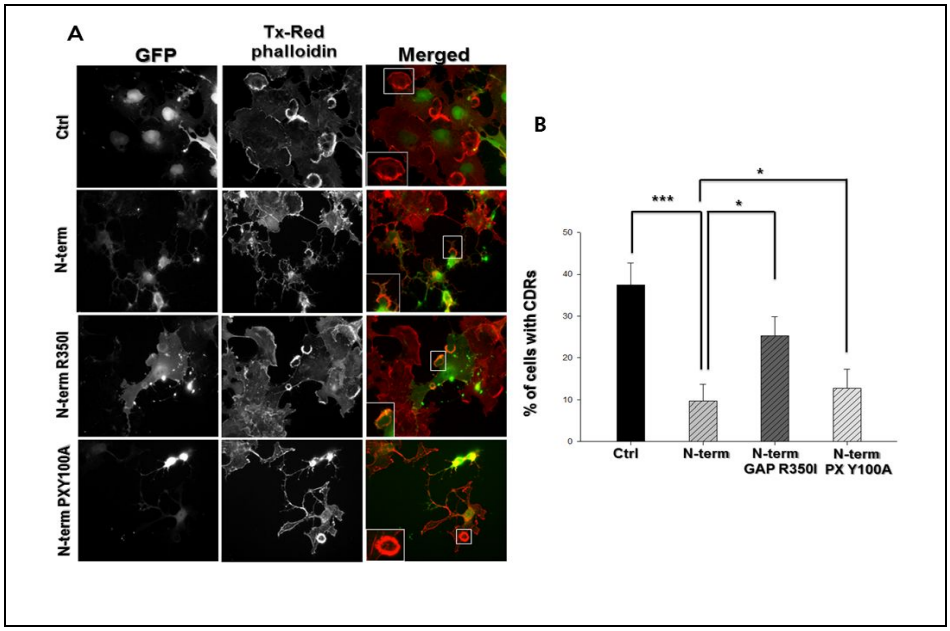

Figure 4. Rho GAP defective mutant has critical role of CDR formation

(A) COS-7 cells were seeded on fibronectin-coated (10μg/ml) coverslip and expressing GFP vector control or GFP-tagged N-term, N-term Rho GAP R350I and N-term PX Y100A were transfected for 24 hr and then starved for 18 hr and stimulated with 400 ng/ml EGF for 5 min at 37℃ and fixed and permeabilized and stained Texas Red-phalloidin. Insert shows CDR structures. (**B**) Presented graph is the percentage of the cells. It shows the cells which have CDR within transfected cells * denotes statistical significance p value<0.05, *** denotes p value< 0.001 using Student's T-test. Error bars denote Standard Error of the Mean (S.E.M)

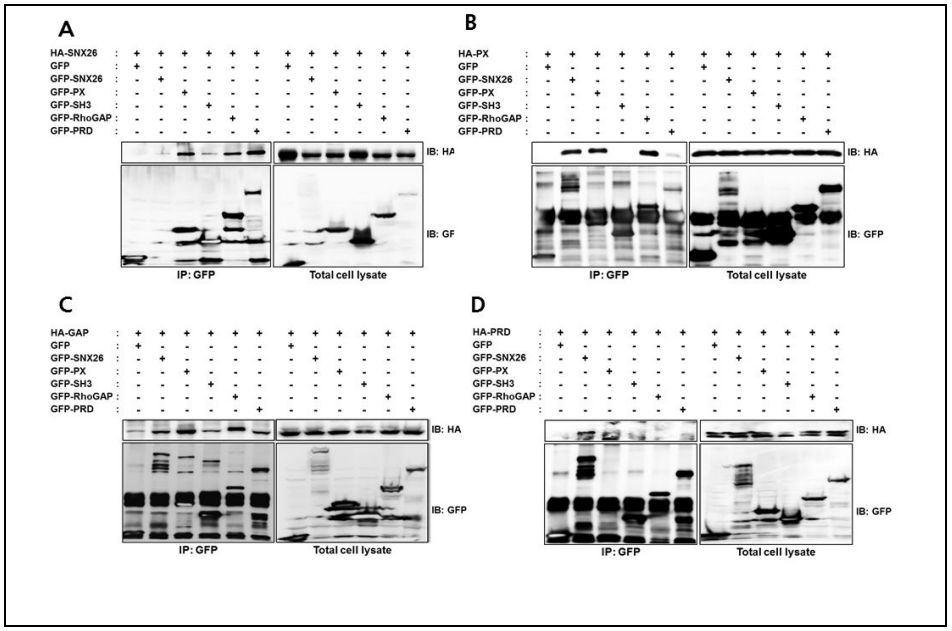

Figure 5. Intra/inter-molecular interaction of SNX26

(A-D) HA- tagged full-length SNX26, PX, Rho GAP, PRD domain were co-transfected into HEK 293T cell with GFP-tagged all fragments of SNX26 (full-length, PX, SH3, Rho GAP and PRD) for 48 hr. Cell lysates were subjected to immunoprecipitation (IP) with GFP and immunoblotting (IB) with anti-HA monoclonal antibody . Total cell lysates were also IB with GFP antibody to detect quantity of each protein

Ⅳ. Discussion

Several studies have been reported that sorting nexin (SNX) family associated with actin-driven endocytic processes. Particularly, it was about the subfamily that has BAR domain, which is known for membrane deforming protein, involved in macropinocytosis [16-18]. SNX26, a member of SNX family, has a Rho GAP domain. Overexpression of SNX26 suppressed neurite outgrowth, and opposite, in expressing a GAP-defective mutant form of SNX26 enhanced in response to nerve growth factor (NGF) in PC12 cells [21]. Rho GAP of SNX26 shows toward Cdc42, is recognized not only help to formation of filopodia structure, but also interact with actin polymerization activator such as N-WASP and Arp2/3 complex in neurons. Likewise several properties of SNX26 have been discovered in neuron [22, 23]. For instance, Rho GAP domain activity controls in neurite outgrowth and extension as well as branching of dendrites and neuronal complexity in the cortex [20, 24].

PX-RICS (known as GC-GAP), one of the RICS isoform, another member of SNX family having Rho GAP domain, also has PX, SH3 and Rho GAP domain. It had highly expression in brain as SNX26 and implicated dendritic morphogenesis as well as cell proliferation in neural and glial cells [25]. However, both of them, little is known about the mechanism, biological function. As pathological disease respects, yet to be identified about SNX26 but in case of PX-RICS, which is associated with risk for schizophrenia and

schizotypal personality traits [26, 27]. Thus, we thought to the possibility that SNX26 is also implicated in this disease.

Src-homology 3 (SH3), is consisted of SNX26, has been worked some characters and functions. One is a peculiarity that binding to PXXP motif region. And preceding studies reported that SH3 domain of other protein may play a role in actin cytoskeleton regulation [28]. But in SNX26 case, this domain did not interaction with PRD (Figure 5). We discreet thought that it is pseudo gene in this result. Though to confirm about character of SH3, some examinations such as protein interaction mediated by proline-rich domain of other protein and SH3 domain of SNX26, CDR formation in expressing only SH3 domain.

It seems that CDR formation reliant largely on complex structure of conserved F-actin and F-actin associated protein. During CDR structures are established, a lot of proteins may assist the formation such as Arp2/3 complex and N-WASP, small family GTPases and actin-capping or binding proteins (cofilin, profilin) [29]. Ruffles or waves are also implicated in bulk fluid-phase endocytosis as these structures can terminate in macropinosome. The morphology and the dynamics of CDR as circular waves have been interesting researchers in the field of mathematical as well as theoretical biology [2]. We also considered that cell-matix interaction is one of the important matters in CDR formation. Several bio-matrix already have been checked on a report [30]. To confirm, we seeded COS-7 cells on many bio-matrixs which are

fibronectin, collagen and Poly-D-Lycine (PDL), fibronectin had hightest number of CDR in same conditions. (data not shown).

In addition, we wondered what CDR formation is that such an important thing in various cells. In our opinion, as already mentioned, conjectured physiological functions of CDR are cell migration as well as internalization and sorting to the newly formed focal adhesion, that might be important for development and wound healing [7].

We showed data that N-terminal fragment of SNX26 effects on the number of CDR (Figure 4), we ascribed this result in not PX but Rho GAP. This our suggestion is based on these data that PX domain bound to Rho GAP domain and had no effect on CDR number, even though it co-localized and interacted with actin. Furthermore, for a reason why the number of CDR in expressing N-term PX Y100A was similar to that of wild type N-terminal. On the other hand, the activity of Rho GAP domain is critical point in CDR formation, indicating that this domain was suppressed through Rho GAP domain. And we tested to know this protein structure via intra-/inter interaction of SNX26. Nevertheless, PX interacted with Rho GAP domain, this data is difficult to describe SNX26 structure. In order to clarify interaction between PX domain and Rho GAP domain, image data is necessary. Therefore, a closer comparison of the effects of Rho GAP in various mammalian cells is needed

In conclusion, our work has showed SNX26 as a GAP effects on CDR

formation also is one of the members in EGFR signaling pathway. Another possibility is that Rho GAP domain may have a one or more roles in by oneself.

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List of Abbreviations

Arp 2/3	Actin related protein 2/3
Cdc 42	cell division cycle 42
CDR	circular dorsal ruffle
COS-7	CV-1 in Origin, and SV40 -7
EGFR	epidermal growth factor receptor
F-actin	filamentous actin
GAP	GTPase activating protein
GDI	guanine nucleotide dissociation inhibitor
GDP	guanosine diphosphate
GEF	guanine nucleotide exchange factor
GFP	green fluorescent protein
GTP	Guanosine-5'-triphosphate
HA	Hydroxylapatite
HEK 293T	Human Embryonic kidney 293T
HGF	hepatocyte growth factor
HRP	Horse Radish Peroxydase
NOMA-GAP	Neurite outgrowth multi adaptor GTPase-activating protein
N-WASP	neural Wiskott-Aldrich syndrome protein
PDGF	platelet-derived growth factor
PI3P	Phospatidylinositol 3-phosphate

PIP_2	Phospatidylinositol (3,4)- bisphosphate
PRD	proline rich domain
PX	Phox homology
Rac 1	Ras-related C3 botulinum toxin substrate 1
RICS	Rho GAP interacting with CIP4 homologues
RTK	receptor tyrosine kinase
SH3	Src homology 3
SNX	sorting nexin
TCGAP	TC10β and Cdc42 GTPase-activating protein

Abstract in Korean (국문 초록)

수 많은 원형질막의 구조는 다양한 물리적 혹은 화학적 자극에 의해 역동적으로 변하게 된다. CDR 은 일시적으로 액틴이 풍부한 고리모양을 일으키며 생성된다. CDR 의 생리학적인 기능으로는 성장인자 수용기의 하향조절과 세포의 이동과 연관되어있다. 그러나 CDR 형성에 대한 정확한 메커니즘은 아직까지 불명확한 상태이다.

SNX26 은 특정적으로 뇌에서 발현하고 있으며 복합적으로 신호를 전달한다고 알려진 Rho GAP domain 을 가지고 있다. COS-7 세포에서 이 Rho GAP 부분을 통해 액틴과 결합하며 CDR 형성을 조절한다. SNX26 의 PX 와 Rho GAP domain 은 액틴과 결합하나 SH3 나 PRD domain 을 통해서는 결합하지 않는다. SNX26 과 이 단백질의 Rho GAP domain 만을 과 발현 시킨 COS-7 세포에서는 섬유질 액틴이 50% 정도 줄어들었다. PX, SH3 그리고 Rho GAP domain 을 포함하고 있는 N-terminal 을 COS-7 세포에 과 발현 시키면 EGF 로 자극을 주었을 때 CDR 형성이 억제된다. 반면에 GAP 으로써 기능을 하지 못하게 하는 Rho GAP R350I 를 발현시킨 세포에서는 EGF 에 의한 CDR 의 수가 증가하였다. 흥미로운 것은 PX 와 PX 가 phosphoinositide 와 결합하지 못하도록 하는 PX Y100A

mutant 는 모두 액틴과 결합을 함에도 불구하고 CDR 형성에 영향을 주지 못했다. 더군다나 SNX 26 은 PX 와 Rho GAP 부분을 통하여 세포간 혹은 세포 내에서 상호작용을 할 가능성을 보였다.

이러한 SNX 26 에 대한 실험은 GAP 으로써 액틴 세포골격을 조절하고 EGF 자극에 의한 CDR 형성을 조절함을 확인할 수 있었다. 뿐만 아니라 세포 간 혹은 세포 내에서의 상호작용에도 역할을 하는 것으로 보여졌다. 이러한 부분들을 모두 종합해 보았을 때 SNX 26 은 세포골격 구조변화를 조절하며 CDR 형성에 있어 EGF 수용기를 통해 Rho 단백질의 신호 전달의 중점적인 역할을 하는 것으로 보여진다.

주요어 : 원형질막 구조변화, 액틴, 세포성장 인자 수용기, 세포 내 과흡입, 세포내 골격구조

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