Biochemical and Biophysical Research Communications 415 (2011) 206–211

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Adaptor protein containing PH domain, PTB domain and leucine zipper (APPL1) regulates the protein level of EGFR by modulating its trafficking

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ARTICLE INFO

*Article history:*
Received 8 October 2011
Available online 19 October 2011

*Keywords:*
APPL1
EGFR
Rab5
Trafficking

ABSTRACT

The EGFR-mediated signaling pathway regulates multiple biological processes such as cell proliferation, survival and differentiation. Previously APPL1 (adaptor protein containing PH domain, PTB domain and leucine zipper 1) has been reported to function as a downstream effector of EGF-initiated signaling. Here we demonstrate that APPL1 regulates EGFR protein levels in response to EGF stimulation. Overexpression of APPL1 enhances EGFR stabilization while APPL1 depletion by siRNA reduces EGFR protein levels. APPL1 depletion accelerates EGFR internalization and movement of EGF/EGFR from cell surface to the perinuclear region in response to EGF treatment. Conversely, overexpression of APPL1 decelerates EGFR internalization and translocation of EGF/EGFR to the perinuclear region. Furthermore, APPL1 depletion enhances the activity of Rab5 which is involved in internalization and trafficking of EGFR and inhibition of Rab5 in APPL1-depleted cells restored EGFR levels. Consistently, APPL1 depletion reduced activation of Akt, the downstream signaling effector of EGFR and this is restored by inhibition of Rab5. These findings suggest that APPL1 is required for EGFR signaling by regulation of EGFR stabilities through inhibition of Rab5.



## 1. Introduction

Upon ligand binding, epidermal growth factor receptor (EGFR) dimerizes and is phosphorylated in its cytoplasmic domain. Phosphorylation in the cytoplasmic domain of EGFR forms docking sites for effector proteins, which activate the downstream signaling pathways such as mitogen-activated protein kinase (MAPK), phosphatidyl inositol 3-kinase (PI3K), Akt, signal transducer and activator of transcription (STAT), protein kinase C (PKC) and phospholipase D (PLD) [1–5]. Upon ligand binding, EGFR is rapidly internalized from the cell surface via several pathways, including clathrin-coated pits [6]. The internalized EGFR is targeted to early endosomes where it is sorted for either the recycling pathway to the plasma membrane or targeted for the degradation pathway in the late endosome/lysosome to terminate the signal [2,6]. The endocytosis of EGFR requires multiple signals, including phosphorylation through its intrinsic tyrosin kinase activity, ubiquitination, and Rab5 [7,8]. Rab5 coordinates endocytosis of surface receptors including EGFR through multiple effectors such as PI3Ks, EEA1 (early endosomal antigen 1), Rabaptin-5/Rabex-5 complex and Rabenosyn-5 [9–12]. The tight regulation of the EGFR-mediated signaling pathway is critical for normal cell functions. Overexpression or dysregulation of EGFR has been associated with many human malignancies such as head and neck cancer, ovarian, cervical, bladder and esophageal cancers [13].

APPL (adaptor protein containing PH domain, PTB domain and leucine zipper) was originally identified as an interacting protein of Akt [14]. It has been reported that APPL1 is involved in the embryonic development of zebrafish by modulating the substrate selectivity of Akt [15]. In addition, APPL1 associates with various transmembrane receptors including DCC (deleted in colorectal cancer, a netrin receptor) [16], TrkA [17], AdipoR (adiponectin receptors) [18], FSHR (follicle-stimulating hormone receptor) [19], and Cdo [20] and plays a key role in the signaling pathway of these receptors. A previous study has reported the role of APPL1 as a downstream effector of EGF-initiated signaling in the nucleus. Upon EGF stimulation, APPL1 interacts with the small GTPase

*Abbreviations:* EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; PI3K, phosphatidyl inositol 3-kinase; STAT, signal transducer and activator of transcription; PKC, protein kinase C; PLD, phospholipase D; EEA1, early endosomal antigen 1; APPL, adaptor protein containing PH domain, PTB domain and leucine zipper; DCC, deleted in colorectal cancer; AdipoR, adiponectin receptors; FSHR, follicle-stimulating hormone receptor; R5BD, Rab5 binding domain of Rabaptin-5; EGF, epidermal growth factor; NGF, nerve growth factor; Rh, rhodamine.

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doi:10.1016/j.bbrc.2011.10.064

Rab5, a key regulator of the transport from the plasma membrane to the early endosomes and this interaction is essential for the regulation of cell proliferation induced by EGF. In the present study, we have investigated an additional function of APPL1 in the EGFR signaling pathway. Because the internalized EGF and APPL1 colocalize in the endosomal compartments and not all APPL1 translocate into nucleus, we hypothesized that APPL1 might regulate EGFR signaling via the endosome-mediated protein trafficking. Here we show that overexpression of APPL1 enhances the stabilization of EGFR proteins while its downregulation through RNA interference reduces the level of EGFR proteins. APPL1-depleted cells exhibit an accelerated internalization and trafficking of EGFR to the perinuclear region, a site for degradation [21], compared to that of control cells. Moreover, APPL1 depletion causes an increase in Rab5 activities and Rab5 inhibition by a dominant-negative form of Rab5 in APPL1-depleted cells restores EGFR protein levels and Akt activation in response to EGF treatment. These findings suggest that APPL1 regulates EGFR signaling via multiple mechanisms including the maintenance of EGFR protein levels by modulation of Rab5 activities.

## 2. Material and methods

### 2.1. Plasmid construction and RNA interference

The human APPL1, Rab5α and Rabaptin-5 genes were amplified by RT-PCR from mRNAs purified from human embryonic kidney fibroblast cells. Full-length APPL1 gene was inserted into mammalian expression vector pCMV6-SRT [22] or pEGFP-C2. To construct the inactive form of Rab5, S34N, site-directed mutagenesis was performed by using following sense and antisense oligonucleotides: 5′-GCTGTTGGCAAAAACAGCCTAGTGCTT-3′ and 5′-AAGCAC TAGGCTGTTTTTGCCAACAGC-3′. Mutation was confirmed by DNA sequencing and mutants were cloned into pCMV6-HA expression vector. For GST-fusion protein of Rab5 binding domain of Rabaptin-5 (R5BD), the amino acid sequence between 739 to 862 of Rabaptin-5 gene was inserted into pGEX-4T-1[23]. Two duplex small interfering RNAs (siRNA) against APPL1 (1; 5′-GGGAGG-CAGGCGTACAAAT and 2; 5′-GCGGGAGAAGUGAAAGUAA) were synthesized from Dharmacon. A negative control scrambled siRNA was purchased from Ambion.

### 2.2. Cell culture and transfection

HeLa cells were grown in DMEM supplemented with 10% fetal bovine serum. For siRNA and expression plasmid transfection in HeLa cells, Neon transfection system (Invitrogen) was used according to the manufacturer's protocol.

### 2.3. Antibodies and chemicals

Generation and purification of the rabbit polyclonal antibody against APPL1 was performed as previously reported [20]. Other antibodies used in this study were: anti-HA (Roche), anti-α-tubulin (Sigma–Aldrich), mouse anti-EGFR (Zymed), rabbit anti-EGFR (Santa Cruz), anti-Akt, anti-phosphorylated Akt (Cell Signaling Technology), anti-EEA1 (BD Biosciences), and FITC-conjugated goat anti-rabbit IgG (ICN). Rhodamine or Alexa Fluor 488-conjugated EGF was purchased from (Invitrogen). The recombinant EGF and Hoechst dye were purchased form Sigma–Aldrich.

### 2.4. Immunoblotting and immunofluorescence

Immunoblot and immunostaining analysis were performed as described previously [24]. Cells were lysed on ice for 30 min in lysis buffer (50 mM Tris–HCl, pH 8.0, 0.1% Triton X-100, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, 1 mM PMSF, 1 mM sodium orthovanadate, 2 mM leupeptin). Proteins were separated by SDS–PAGE and transferred. After blocking, membranes were probed with appropriate antibodies and detected with ECL system (GE Healthcare). The densitometric quantification was performed using LAS-3000 and analyzed with Image Gauge image analysis software (Fuji Photo Film). For immunostaining, HeLa cells were fixed with 4% paraformaldehyde in PBS, followed by washing, blocking, and a successive hybridization with an EEA1 antibody and Rhodamine-conjugated anti-mouse antibody. Confocal microscopy analyses were carried out by using an LSM510 META (Carl Zeiss).

### 2.5. EGFR internalization assay

HeLa cells transfected with either the control scrambled or siRNA for APPL1 were incubated with 100 ng/ml of EGF for the indicated time period. Cells were washed with ice-cold PBS followed by incubation with a solution containing 0.5 mg/ml sulfo-NHS-SS-biotin (Thermo Scientific) at 4 °C for 30 min. The reaction was quenched by washing the cells with ice-cold TBS. After sonication in a lysis buffer (50 mM Tris, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 0.1% Triton X-100 pH 7.4), lysates were incubated with immobilized streptavidin-beads for 1 h and washed several times with lysis buffer. After brief centrifugation, precipitated proteins were analyzed by immunoblotting.

### 2.6. Vesicle movement

HeLa cells were observed using LSM 510 Meta equipped with climate-controlled stage. Cells transfected either with the control scrambled siRNA or APPL1 siRNA were starved for 12 h followed by incubation with 100 ng/ml of EGF-488. Time-lapse imaging was started 5 min after EGF stimulation with an interval of 2 s for 10 min. Tracking of EGF-488 containing endosomes was conducted using the Manual Tracking Image J plug-in (NIH).

## 3. Results

### 3.1. APPL1 regulates the protein level of EGFR

To investigate the possibility that APPL1 might regulate EGF signaling in endosomal compartments via regulation of EGFR stabilities, we have altered APPL1 levels by overexpression or knockdown and analyzed EGFR protein levels. HeLa cells were transfected with the control or SRT-tagged human APPL1 expression vectors. Forty-eight hours later, cells were treated with EGF and harvested at various time points and as shown in Fig. 1A and B, the EGFR protein level was starkly reduced at 60 min of EGF treatment and almost disappeared at 90 min in the control cells. In APPL1 overexpressing cells, the level of EGFR protein was maintained longer and it was significantly higher at 90 min of EGF treatment, relative to the control cells. Next, HeLa cells were transfected with two different siRNAs against APPL1 or a control siRNA and 48 h after cells were treated with EGF for various times followed by immunoblot analysis. APPL1 was almost completely depleted by both APPL1 siRNAs. In later experiments, we have used APPL1 siRNA1. Both APPL1-depleted cells exhibited a strong reduction in EGFR protein levels already at 30 min of EGF treatment which was comparable to the EGFR protein level at 60 min treatment in control cells (Fig. 1C and D). These data suggest that APPL1 is required to maintain EGFR protein levels in response to EGF treatment.

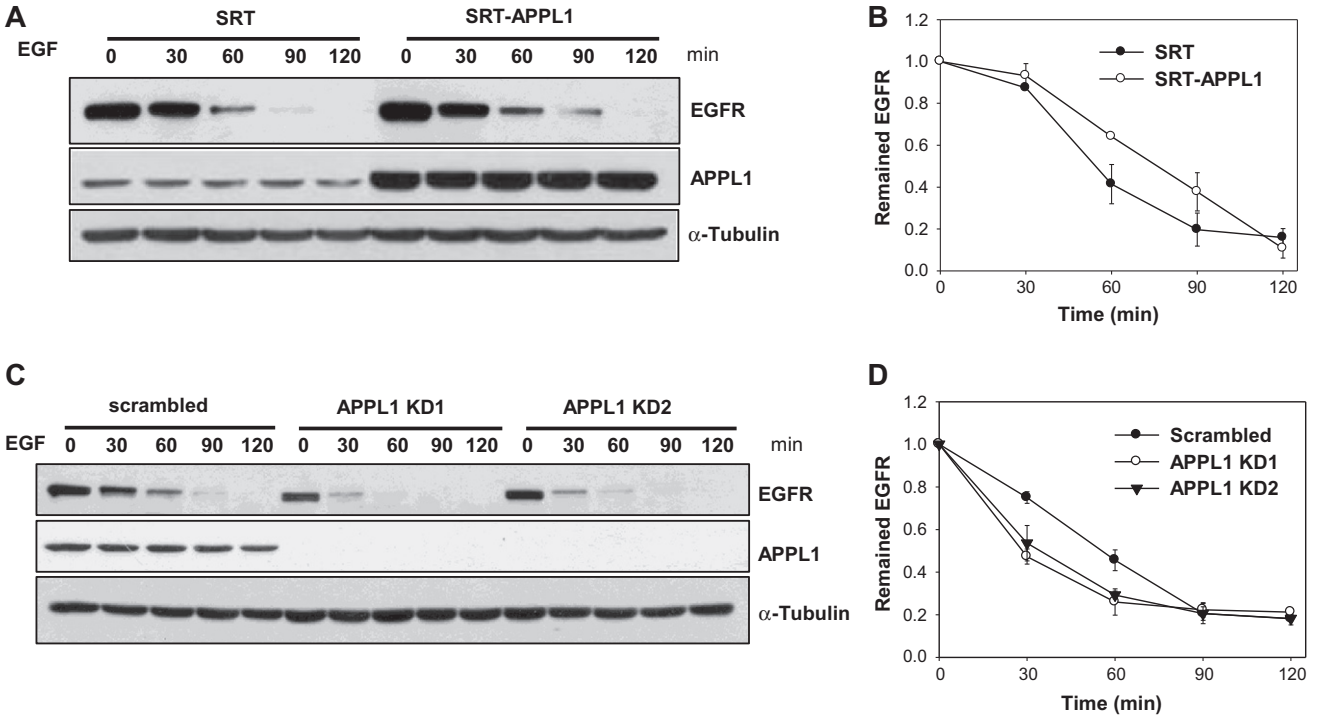



**Fig. 1.** APPL1 regulates EGFR protein levels. (A) HeLa cells transfected either with control SRT or SRT-APPL1 expression vector were serum-starved for 12 h and then stimulated with 100 ng/ml of EGF for indicated times. Cell lysates were immunoblotted with antibodies to EGFR, APPL1 or α-tubulin as a loading control. (B) Relative densitometric analysis of remaining EGFR from similar experiments shown in panel (A). The value reflect the mean ± S.E. of three independent experiments. (C) HeLa cells were transfected either with scrambled siRNA or siRNAs against APPL1. Twenty-four hours later, cells were treated and analyzed as described in (A). (D) Relative densitometric analysis of remaining EGFR from similar experiments shown in panel (C). The value reflect the mean ± S.E. of three independent experiments.

### 3.2. APPL1 regulates EGFR trafficking

Upon EGF treatment, EGFR internalization is the first step in its trafficking and degradation, which is critical for determining the duration of EGF signaling. Therefore, we tested the possibility that APPL1 may modulate EGFR internalization thereby affecting EGFR protein stability. To do so, we have first assessed whether internalization of EGFR is affected by APPL1 depletion, HeLa cells transfected with the control scrambled or APPL1 siRNA were incubated with EGF for the indicated time periods. Cells were then incubated with sulfo-NHS-SS-biotin, followed by washing, pulling down with streptoavidin beads and immunoblotting for the surface-bound biotinylated EGFR. After 10 min of EGF treatment, the remaining EGFR at the cell surface was reduced to 50% and was further decreased to 40% at 20 min of incubation in control cells, relative to the untreated control. This process was accelerated in APPL1-depleted cells. At 10 min of EGF treatment, the surface EGFR decreased to 10% in APPL1-depleted cells, relative to the untreated control (Fig. 2A and B).

To further characterize the role of APPL1 in regulation of the EGFR protein stability, we have analyzed the movement of EGFR proteins from the cell surface to the early endosomal compartments by tracking a fluorescence-labeled EGF. HeLa cells were transfected with either the control scrambled siRNA or APPL1 siRNA and 48 h later these cells were treated with Alexa Fluor 488-conjugated EGF (EGF-488) for the indicated times, followed by immunostaining for EEA1, an early endosomal marker (red). In the control cells, after 15 min the majority of EGF-488 positive and EEA1-positive vesicles were found in the perinuclear region. In APPL1-depleted cells, EGF-488 colocalized with EEA1-positive endosomes in the perinuclear region after 10 min and the signal of EGF-488 was almost disappeared after 15 min (Fig. 3A). We next examined the effect of APPL1 overexpression on the intracellular

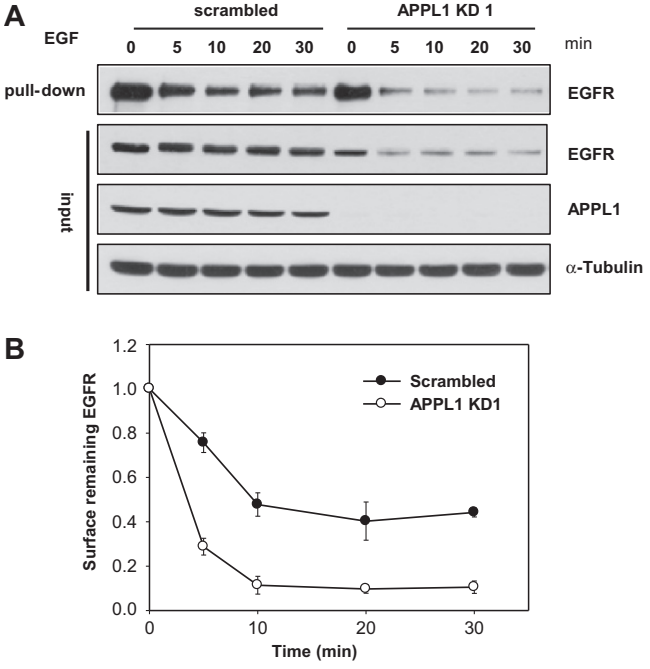
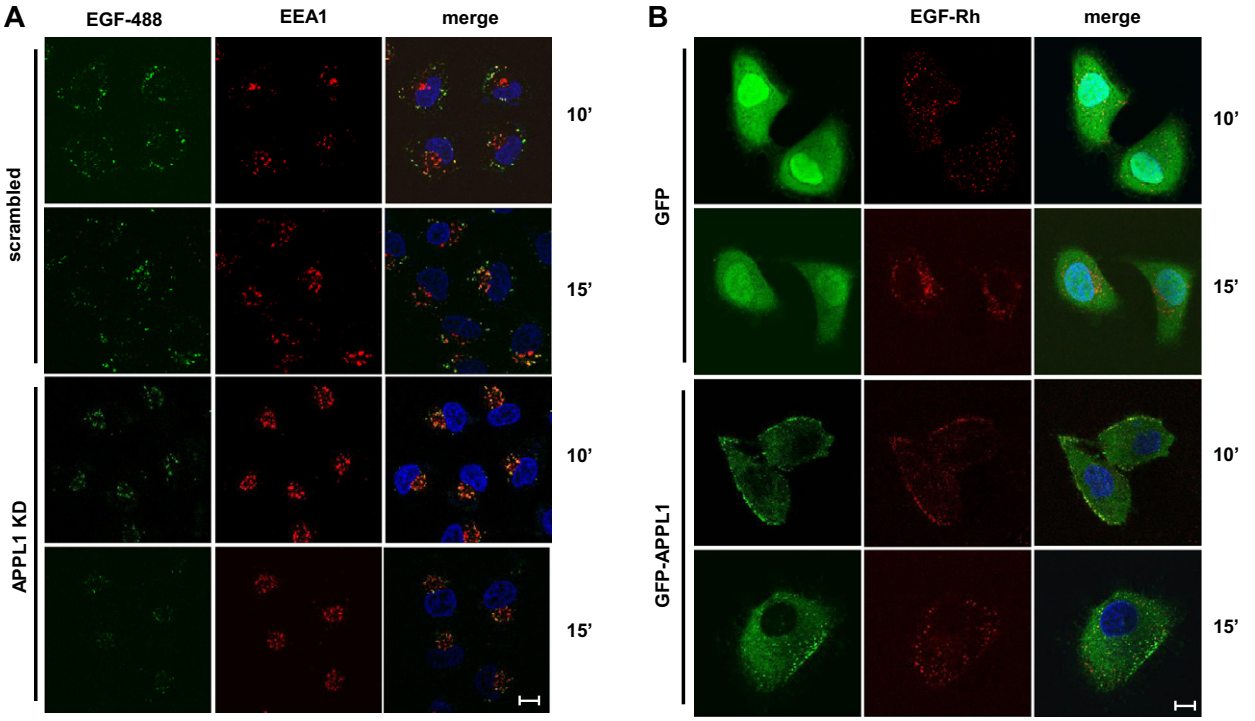



**Fig. 2.** APPL1 depletion causes rapid EGFR internalization. (A) HeLa cells transfected either with scrambled siRNA or APPL1 siRNA1 were incubated with EGF for indicated times and the unbound ligand was washed out with PBS. The surface proteins were labeled with biotin and precipitated with streptavidin-beads followed by immunoblotting with antibodies against EGFR, APPL1, or α-tubulin. (B) The surface remaining EGFR was quantified as the ratio of the biotin-labeled EGFR at the beginning to that of EGF stimulated at each time points. The results represent the mean ± S.E. of three independent experiments.

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| | average velocity (nm/sec) |
|---|---|
| Scrambled | 34.1 ± 11.8 (n=54) |
| APPL1 KD | 51.2 ± 16.4 (n=53) |

**Fig. 3.** APPL1 regulates trafficking of EGFR protein. (A) HeLa cells were transfected either with scrambled siRNA or APPL1 siRNA. Twenty-four hours later, cells were serum-starved for 12 h, and treated with 100 ng/ml of EGF-488 (green) for the indicated time periods. Cells were then fixed and immunostained with anti-EEA1 antibody (red). Cell nuclei were visualized by staining with Hoechst reagent. Scale bar, 10 μm. (B) HeLa cells were transfected either with the control GFP or GFP-APPL1 expression vector. Twenty-four hours later, cells were serum-starved for 12 h, and treated with 100 ng/ml of EGF-Rh (red) for the indicated time periods followed by fixation and confocal microscopy. Scale bar, 10 μm. (C) Table summarizing velocities of EGF/EGFR vesicles in control and APPL1-depleted cells.

movement of EGF/EGFR proteins. HeLa cells were transfected with either the control GFP or GFP-tagged APPL1 (APPL1-GFP) and cells were treated with rhodamine-conjugated EGF (EGF-Rh) for the indicated time period. In the control cells, EGF-Rh-positive vesicles were scattered throughout the cytoplasm and a small fraction was found in the perinuclear region at 10 min after the EGF-Rh treatment. However, almost all of these vesicles were found in the perinuclear compartment after 15 min (Fig. 3B). In contrast, a large proportion of EGF-Rh still remained in the close proximity of the plasma membrane at 10 min of treatment in APPL1-GFP overexpressing cells, where it colocalized largely with APPL1-GFP. At 15 min, EGF-Rh-positive vesicles were still observed in the cytoplasm and a fraction of EGF-Rh-positive vesicles were detected in the perinuclear compartment in APPL1-GFP transfected cells (Fig. 3B). We then measured the velocity of EGF-containing vesicles by using time-lapse microscopy. HeLa cells transfected either with the control scrambled siRNA or APPL1 siRNA were starved for 12 h followed by incubation with EGF-488. The velocity of EGF-containing vesicles was increased about 1.5 times in APPL1-depleted cells, compared to that of the control cells (Fig. 3C). These data demonstrate that APPL1 regulates the trafficking of EGF/EGFR from cell surface to early endosomal compartments.

### 3.3. APPL1 inhibits the activation of RAB5

It was previously reported that APPL1 interacts with the activated Rab5 and Rab5 is required for both internalization and trafficking of EGFR to endosomal compartments leading to EGFR degradation [25,26]. Therefore we assessed whether APPL1 regulates EGFR trafficking via modulation of Rab5 activities. To do so, the control and APPL1-depleted HeLa cells were transfected with a HA-tagged Rab5 and treated with EGF for 5 min. We then analyzed Rab5 activities in the control and APPL1-depleted cells by using a purified GST-fusion protein of the Rab5-binding domain of Rabaptin-5 (R5BD). Lysates of the control and APPL1-depleted HeLa cells were incubated with R5BD followed by the pulldown with glutathione-agarose beads and immunoblotting with antibodies against Rab5 (HA). Five minutes after EGF stimulation, Rab5 binding to R5BD increased about two folds in control cells (Fig. 4A and B). In APPL1-depleted cells Rab5 protein levels bound to R5BD were increased about two folds under the basal condition without EGF treatment and it further increased about 1.5 folds at 5 min of EGF treatment, compared to the control cells (Fig. 4A and B). These data suggested that APPL1 depletion led to enhanced Rab5 activities and the increased activity of Rab5 might cause a decrease in EGFR protein stability in APPL1-depleted cells. Therefore, we tested whether Rab5 inhibition could restore the EGFR protein stability in APPL1-depleted cells. To inhibit Rab5, we have employed a dominant negative form of Rab5 (S34N). The level of EGFR protein was strongly increased in S34N transfected control cells and enhanced Akt activation, a downstream signaling effector of EGFR correlated well with this increase of EGFR protein levels. Furthermore Rab5 inhibition in APPL1 knockdown cells resulted in maintaining EGFR levels until 60 min after EGF treatment. The con-

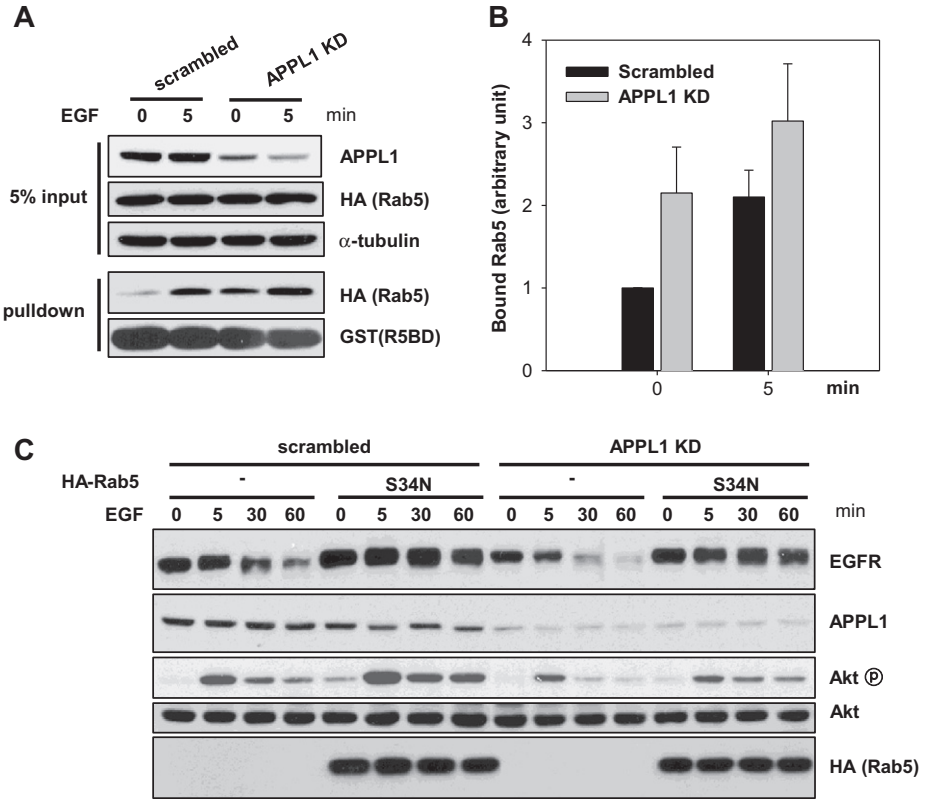


**Fig. 4.** APPL1 regulates Akt phosphorylation through the activity of Rab5 (A) HeLa cells were cotransfected either with scrambled siRNA or siRNA against APPL1 plus the HA tagged-Rab5 expression vector. Cell lysates were incubated with GST-R5BD and pulled down with glutathione-beads followed by immunoblotting with antibodies to HA or GST. As control, 5% of input lysates were analyzed by immunoblotting with antibodies to APPL1, HA or α-tubulin. (B) R5BD-bound Rab5 levels were quantified by densitometry in comparison with the level of R5BD-pulldown Rab5 in the untreated control cells which was set to 1. These values are means of two independent experiments. (C) HeLa cells were transfected either with scrambled siRNA or APPL1 siRNA along with S34N expression vector. Twenty-four hours later, cells were serum-starved for 12 h, and treated with 100 ng/ml of EGF for the indicated time periods. Cell lysates were subjected to immunoblotting with antibodies to EGFR, APPL1, phosphorylated-Akt, Akt or HA.

trol vector transfected APPL1-depleted cells exhibited reduced Akt activation, compared to that of the control cells and it is only readily detected at 5 min of EGF stimulation. In contrast, the active form of Akt was detected until 60 min after EGF stimulus in S34N-overexpressing, APPL1-depleted cells which correlated well with the maintenance of EGFR protein. Interestingly however the initial Akt activation at 5 min of EGF stimulation in these cells was not restored to the level of the control cells (Fig. 4C). Taken together, these data suggest that APPL1 regulates EGFR protein stability via modulation of Rab5 activation.

## 4. Discussion

Upon EGF stimulation, EGFR associates with other molecules to initiate signal transmission and regulates cell proliferation and differentiation. Some of associating molecules are involved in terminating signal transmission via internalization and ubiquitination of EGFR, followed by sorting of EGFR to lysosomes for its degradation. Uncontrolled activation or delayed degradation of the EGFR protein has been linked to progression of human malignancies [13]. In contrast, to receive and relay an optimal level of signals for cell survival or proliferation, sufficient amounts of receptors should be stably present on the plasma membrane available for ligand binding which will secure the proper duration of signal generation and transmission to ensure gene activation. In addition, too rapid internalization and degradation of receptors would also be deleterious to cellular homeostasis. Upon EGF stimulus, APPL1 interacts with Rab5 and translocates into nucleus regulating DNA synthesis required for cell proliferation [27]. The fact that the majority of APPL1 proteins were observed in cytoplasm, not in the nucleus after EGF treatment led us to assess its role in endosomal compartments which is not well understood. In this study, we demonstrate that overexpression of APPL1 reduces EGFR degradation induced by EGF stimulation, whereas APPL1 depletion results in decreased EGFR protein levels, suggesting that APPL1 is required for the maintenance of EGFR protein levels. One major mechanism by which APPL1 regulates the EGFR protein stability is by modulating the Rab5 activation. APPL1 depletion causes an increase in Rab5 activities and Rab5 inhibition by expression of a dominant negative form of Rab5 restores EGFR protein levels in APPL1-depleted cells, relative to the control cells. APPL1 has been reported to be an effector protein of Rab5 [27]. It is possible that APPL1 functions as both an downstream effector as well as a regulator of Rab5 activities, like RN-tre which works as both a RabGAP and a Rab5 effector [28]. Since APPL1 is known to associate with the GTP-bound form of Rab5 and inhibit Rab5 activation we have analyzed whether APPL1 possesses a Rab5-GAP activity thereby regulating Rab5 activity directly but failed to find it (unpublished data). Currently we do not know how APPL1 regulates Rab5 activity. It is likely that APPL1 may regulate Rab5 activities indirectly by modulating activities of RabGAPs or RabGEFs.

It has been reported that APPL1 acts as a signaling molecule in Akt activation by several ligands such as adiponectin, EGF, NGF, insulin [17,18,27,29,30]. Consistently Akt activities were reduced in APPL1 depleted cells correlating with the reduced level of EGFR and Rab5 inhibition restored the level of EGFR proteins and the duration of Akt activation. Interestingly however the initial activa-

tion of Akt at 5 min of the EGF stimulation was not restored by Rab5 inhibition, even though EGFR levels were restored to the control level. These data suggest that APPL1 regulates Akt activation at the initial phase of EGF treatment independently of Rab5. Currently it is unclear how APPL1 regulates the early phase of the EGF-mediated Akt activation. APPL1 has been shown to be involved in the substrate specificity of Akt by regulating compartmentalization of Akt in specific vesicles. Therefore, it is possible that APPL1 may regulate Akt activation at the early phase of EGF stimulation via Rab5-independent compartmentalization in specific vesicles. In this report, we have demonstrated that APPL1 is required for maintaining EGFR proteins levels and the downstream signal transmission in response to EGF stimulus. The APPL1-mediated EGFR stabilization involves inhibition of Rab5 activities via unknown mechanisms. Hyperactivation of EGFR-mediated signaling has been associated with numerous malignant conditions in humans. As APPL1 appears to be important for maintenance of this signal, we believe that APPL1 is a good candidate to target for the treatment of cancers caused by dysregulation of EGF signaling.

## Acknowledgments

This work was supported by grants from the 2001 Good Health R & D Project of the Ministry of Health (01-PJ3-PG6-01GN12-0001) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0005053).

## References

[1] R.N. Jorissen, F. Walker, N. Pouliot, T.P. Garrett, C.W. Ward, A.W. Burgess, Epidermal growth factor receptor: mechanisms of activation and signalling, Exp. Cell Res. 284 (2003) 31–53.
[2] C. Le Roy, J.L. Wrana, Clathrin-and non-clathrin-mediated endocytic regulation of cell signalling, Nat. Rev. Mol. Cell Biol. 6 (2005) 112–126.
[3] G. Carpenter, The EGF receptor: a nexus for trafficking and signaling, Bioessays 22 (2000) 697–707.
[4] S. Grant, L. Qiao, P. Dent, Roles of ERBB family receptor tyrosine kinases downstream signaling pathways in the control of cell growth and survival, Front Biosci. 7 (2002) d376–d389.
[5] J. Schlessinger, Common and distinct elements in cellular signaling via EGF and FGF receptors, Science 306 (2004) 1506–1507.
[6] H. Waterman, Y. Yarden, Molecular mechanisms underlying endocytosis and sorting of ErbB receptor tyrosine kinases, FEBS Lett. 490 (2001) 142–152.
[7] I. Dikic, Mechanisms controlling EGF receptor endocytosis and degradation, Biochem. Soc. Trans. 31 (2003) 1178–1181.
[8] X. Chen, Z. Wang, Regulation of intracellular trafficking of the EGF receptor by Rab5 in the absence of phosphatidylinositol 3-kinase activity, EMBO Rep. 2 (2001) 68–74.
[9] S. Christoforidis, M. Miaczynska, K. Ashman, M. Wilm, L. Zhao, S.C. Yip, M.D. Waterfield, J.M. Backer, M. Zerial, Phosphatidylinositol-3-OH kinases are Rab5 effectors, Nat. Cell Biol. 1 (1999) 249–252.
[10] A. Simonsen, R. Lippe, S. Christoforidis, J.M. Gaullier, A. Brech, J. Callaghan, B.H. Toh, C. Murphy, M. Zerial, H. Stenmark, EEA1 links PI (3) K function to Rab5 regulation of endosome fusion, Nature 394 (1998) 494–498.
[11] R. Lippe, M. Miaczynska, V. Rybin, A. Runge, M. Zerial, Functional synergy between Rab5 effector Rabaptin-5 and exchange factor Rabex-5 when physically associated in a complex, Mol. Biol. Cell 12 (2001) 2219.
[12] E. Nielsen, S. Christoforidis, S. Uttenweiler-Joseph, M. Miaczynska, F. Dewitte, M. Wilm, B. Hoflack, M. Zerial, Rabenosyn-5, a novel Rab5 effector, is complexed with hVPS45 and recruited to endosomes through a FYVE finger domain, J. cell Biol. 151 (2000) 601.
[13] P.M. Harari, Epidermal growth factor receptor inhibition strategies in oncology, Endocr. Relat. Cancer 11 (2004) 689–708.
[14] Y. Mitsuuchi, S.W. Johnson, G. Sonoda, S. Tanno, E.A. Golemis, J.R. Testa, Identification of a chromosome 3p14. 3-21.1 gene, APPL, encoding an adaptor molecule that interacts with the oncoprotein-serine/threonine kinase AKT2, Oncogene 18 (1999) 4891–4898.
[15] A. Schenck, L. Goto-Silva, C. Collinet, M. Rhinn, A. Giner, B. Habermann, M. Brand, M. Zerial, The endosomal protein Appl1 mediates Akt substrate specificity and cell survival in vertebrate development, Cell 133 (2008) 486–497.
[16] J. Liu, F. Yao, R. Wu, M. Morgan, A. Thorburn, R.L. Finley, Y.Q. Chen, Mediation of the DCC apoptotic signal by DIP13a, J. Biol. Chem. 277 (2002) 26281–26285.
[17] D.C. Lin, C. Quevedo, N.E. Brewer, A. Bell, J.R. Testa, M.L. Grimes, F.D. Miller, D.R. Kaplan, APPL1 associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction, Mol. Cell Biol. 26 (2006) 8928–8941.
[18] X. Mao, C.K. Kikani, R.A. Riojas, P. Langlais, L. Wang, F.J. Ramos, Q. Fang, C.Y. Christ-Roberts, J.Y. Hong, R.Y. Kim, F. Liu, L.Q. Dong, APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function, Nat. Cell Biol. 8 (2006) 516–523.
[19] C.A. Nechamen, R.M. Thomas, J.A. Dias, APPL1, APPL2, Akt2 and FOXO1a interact with FSHR in a potential signaling complex, Mol. Cell. Endocrinol. 260-262 (2007) 93–99.
[20] G.U. Bae, J.R. Lee, B.G. Kim, J.W. Han, Y.E. Leem, H.J. Lee, S.M. Ho, M.J. Hahn, J.S. Kang, Cdo interacts with APPL1 and activates AKT in myoblast differentiation, Mol. Biol. Cell 21 (2010) 2399.
[21] C. Futter, Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes, J. Cell Biol. 132 (1996) 1011–1023.
[22] J.R. Lee, Y.Y. Chang, M.J. Hahn, Development of a new epitope tag recognized by a monoclonal antibody to Rickettsia typhi, Biotechniques 31 (2001) 541–545.
[23] J. Liu, D. Lamb, M.M. Chou, Y.J. Liu, G. Li, Nerve growth factor-mediated neurite outgrowth via regulation of Rab5, Mol. Biol. Cell 18 (2007) 1375.
[24] S.J. Lee, J.R. Lee, H.S. Hahn, Y.H. Kim, J.H. Ahn, C.D. Bae, J.M. Yang, M.J. Hahn, PIAS1 interacts with the KRAB zinc finger protein, ZNF133, via zinc finger motifs and regulates its transcriptional activity, Exp. Mol. Med. 39 (2007) 450–457.
[25] X. Chen, Z. Wang, Regulation of epidermal growth factor receptor endocytosis by wortmannin through activation of Rab5 rather than inhibition of phosphatidylinositol 3-kinase, EMBO Rep. 2 (2001) 842–849.
[26] J.L. Dinneen, B.P. Ceresa, Expression of dominant negative rab5 in HeLa cells regulates endocytic trafficking distal from the plasma membrane, Exp. Cell Res. 294 (2004) 509–522.
[27] M. Miaczynska, S. Christoforidis, A. Giner, A. Shevchenko, S. Uttenweiler-Joseph, B. Habermann, M. Wilm, R.G. Parton, M. Zerial, APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment, Cell 116 (2004) 445–456.
[28] L. Lanzetti, A. Palamidessi, L. Areces, G. Scita, P.P. Di Fiore, Rab5 is a signalling GTPase involved in actin remodelling by receptor tyrosine kinases, Nature 429 (2004) 309–314.
[29] T. Varsano, M.Q. Dong, I. Niesman, H. Gacula, X. Lou, T. Ma, J.R. Testa, J.R. Yates Iii, M.G. Farquhar, GIPC Is Recruited by APPL to Peripheral TrkA Endosomes and Regulates TrkA Trafficking and Signaling?, Mol Cell. Biol. 26 (2006) 8942–8952.
[30] T. Saito, C.C. Jones, S. Huang, M.P. Czech, P.F. Pilch, The interaction of Akt with APPL1 is required for insulin-stimulated Glut4 translocation, J. Biol. Chem. 282 (2007) 32280–32287.