IQGAP1 Scaffolds EGFR Interactions with Downstream Kinases

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Written by

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Abstract

IQ-motif-containing GTPase-activating protein 1 (IQGAP1) is a multi-domain scaffolding protein that binds to several different proteins and is involved in many signaling pathways. It has been proposed that IQGAP1 participates in the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP_2) to form the signaling lipid phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) by interacting with phosphatidylinositol-3-OH kinase (PI3K). The generation of PIP_3 recruits the serine-threonine kinase Akt1 to the plasma membrane, where it is phosphorylated and activated. This represents an upstream step of the PI3K/Akt/mTOR pathway, which regulates cell survival, proliferation, and cell cycle progression. This signaling pathway is initiated when the epidermal growth factor receptor (EGFR) binds to its cognate ligand, EGF. Both *AKT1* and *EGFR* have been classified as oncogenes due to various mutations and increased activity found in certain forms of cancer. It is known that IQGAP1 interacts with each protein individually but it is unknown if IQGAP1 scaffolds Akt1 and EGFR. In this study, we investigated the binding of IQGAP1 to both proteins in the HeLa cell line using Fluorescence Lifetime Imaging Microscopy (FLIM). We confirmed that Akt1 and EGFR do interact in the presence of IQGAP1. Additionally, an increased interaction between PIP_3, studied using the sensor PH domain of Akt1, and EGFR was observed upon stimulation with EGF. This interaction was found to be mediated through IQGAP1 scaffolding, which suggests that it plays a key role in modulating the PI3K/Akt/mTOR signaling pathway's response to pro-migratory stimuli such as EGF.

Introduction

Scaffolding Proteins
In mammalian cells, many protein molecules interact with each other and transmit signaling information (Good, Zalatan, and Lim, 2011). Specific and efficient interactions of these proteins are essential to a cell's function and survival. Through billions of years of evolution, cells have developed organizational systems to process the activity of signaling proteins effectively. One commonly-known form of compartmentalization is a cellular organelle, which can be highly specialized to host only certain proteins in a defined location in the cell (Dallarosa, 2018). Over 20 years ago, scaffolding proteins were discovered and were identified as spatial coordinators of proteins involved in pathways and networks (Dallarosa, 2018). To achieve the physical assembly of pathway proteins, scaffolding proteins have multiple domains or motifs (Dallarosa, 2018).

History of the Discovery of IQGAP1
Mammalian IQGAP1 was first identified and sequenced at Massachusetts General Hospital Cancer Center and Orthopedic Research Laboratories in 1994 (Weissbach, et al., 1994). This study revealed that IQGAP1 shares 23% of its sequence with the RasGAP Sar1 protein found in *Schizosaccharomyces pombe*. Human IQGAP2 was identified in 1996 and shares 62% of its sequence with human IQGAP1 (Briggs and Sacks, 2003). Finally, IQGAP3 was discovered in 2007; while its sequence is known and has similar domains as its isoforms, many of its functions remain unconfirmed (White, et al., 2011). These three IQGAPs have defined locations within the human body, with IQGAP1 expressed ubiquitously in all cells, IQGAP2 present predominately in the liver, and IQGAP3 in the brain, liver, colon, small intestine, and lungs (White, et al., 2011).

Background of IQGAP1
IQ motif containing GTPase Activating Proteins (IQGAP) are a family of scaffolding proteins that are key facilitators of cytoskeleton structure, cellular migration, intracellular signaling, and intercell interactions in numerous eukaryotic organisms (Briggs and Sacks, 2003). IQGAP1 has been extensively researched since its initial discovery and is an influential scaffolding protein in mammalian cells, interacting with a large number of other proteins (Abel, et al., 2015 and Bañón-Rodríguez, et al., 2014). IQGAP1 homologs have been identified in various organisms, including yeast, roundworms, frogs, and mice (Briggs and Sacks, 2003). In fact, human and mouse IQGAP1 proteins have 96% similar amino acid sequences (Abel, et al., 2015). Furthermore, the IQGAP family is evolutionarily conserved, which suggests that these proteins mediate essential and primordial cellular functions.

Mammalian IQGAP1 is the best-characterized member of this family, and its various domains have been intensively investigated. IQGAP1 is 190kDa long and possesses six distinct domains (Dallarosa, 2018). A calponin homology domain (CHD) is located at the amino terminus; next is six repeats of a 50-amino acid sequence, which is known as the repeats or coiled-coil domain. This is followed by a WW domain, which is named after the two conserved tryptophan residues

(Samson, et al., 2017 and Bañón-Rodríguez, et al., 2014). Following this are four repeats of the IQ motif, which binds to calmodulin, a calcium signaling messenger protein (Briggs and Sacks, 2003). A RasGAP-Related Domain (GRD) precedes the RasGAP C-terminal domain (RGCT) at the carboxyl terminus (Samson, et al., 2017). Although IQGAP1 does not possess GTPase activating protein activity, it is able to bind and stabilize the active forms of Rho family GTPases such as Rac1 and Cdc42 (Samson, et al., 2017).
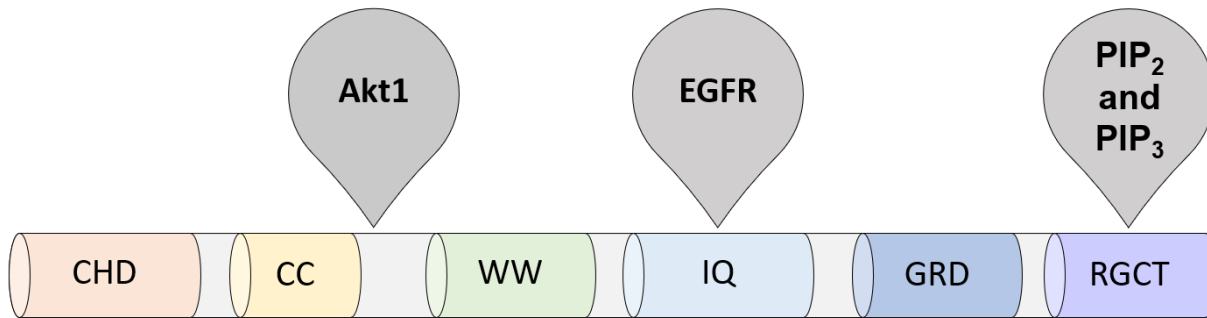

Figure 1: IQGAP1 Protein Domains with Akt1 and EGFR Binding Sites. IQGAP1 has six distinct domains that bind to a variety of binding partners, including β-actin, PI3K, talin, Cdc42, Akt1, and EGFR. IQGAP1 has been found to bind to a large number of different proteins, facilitating cell-to-cell adhesion, cell migration, signal transduction, and actin remodeling (Bañón-Rodríguez, et al., 2014). Akt1 binds to IQGAP1 near the CC domain, with the downstream target FoxO1 directly binding to this domain once phosphorylated by Akt1. This interaction induces a conformational change that forces IQGAP1 to dissociate from Ras pathway proteins, including Raf, MEK, and ERK (Choi and Anderson, 2017). In fact, for higher concentrations of IQGAP1 in cells, the PI3K signaling pathway is scaffolded while the Ras pathway is scaffolded with lower concentrations of IQGAP1 (Choi and Anderson, 2017). EGFR binds to IQGAP1 in the IQ motif region and it has been proposed that this interaction promotes mitotic spindle orientation and distribution of IQGAP1 in the basolateral membrane (Bañón-Rodríguez, et al., 2014). Additionally, PIP_2 and PIP_3 bind to the RGCT domain of IQGAP1 (Choi, et al., 2013). With its multiple domains, IQGAP1 is able to bind to a variety of proteins and simultaneously form multiprotein complexes.

PI3K/Akt/mTOR Signaling Pathway
The PI3K/Akt/mTOR signaling pathway regulates cell survival processes, including growth, metabolism, and apoptosis (Dallarosa, 2018). Phosphoinositide 3-Kinases (PI3Ks) phosphorylate the 3-hydroxyl group on phosphatidylinositols, which are cell membrane phospholipids that mediate the functions of regulatory proteins (Balla, 2013). Following activation from growth factors and other regulators, PI3K phosphorylates PIP_2 to generate PIP_3 (Mahadevan, et al., 2008). PIP_3 is a crucial signaling phospholipid that is synthesized in the PI3K pathway in the presence of IQGAP1 (Dallarosa, 2018). On the other hand, PIP_3 levels are negatively regulated by PTEN, which is classified as a tumor suppressor protein. Following its generation, PIP_3 facilitates the activation of Akt1 by PDK1, which further signals downstream pathway components linked to metabolism, survival, and differentiation (Balla, 2013).
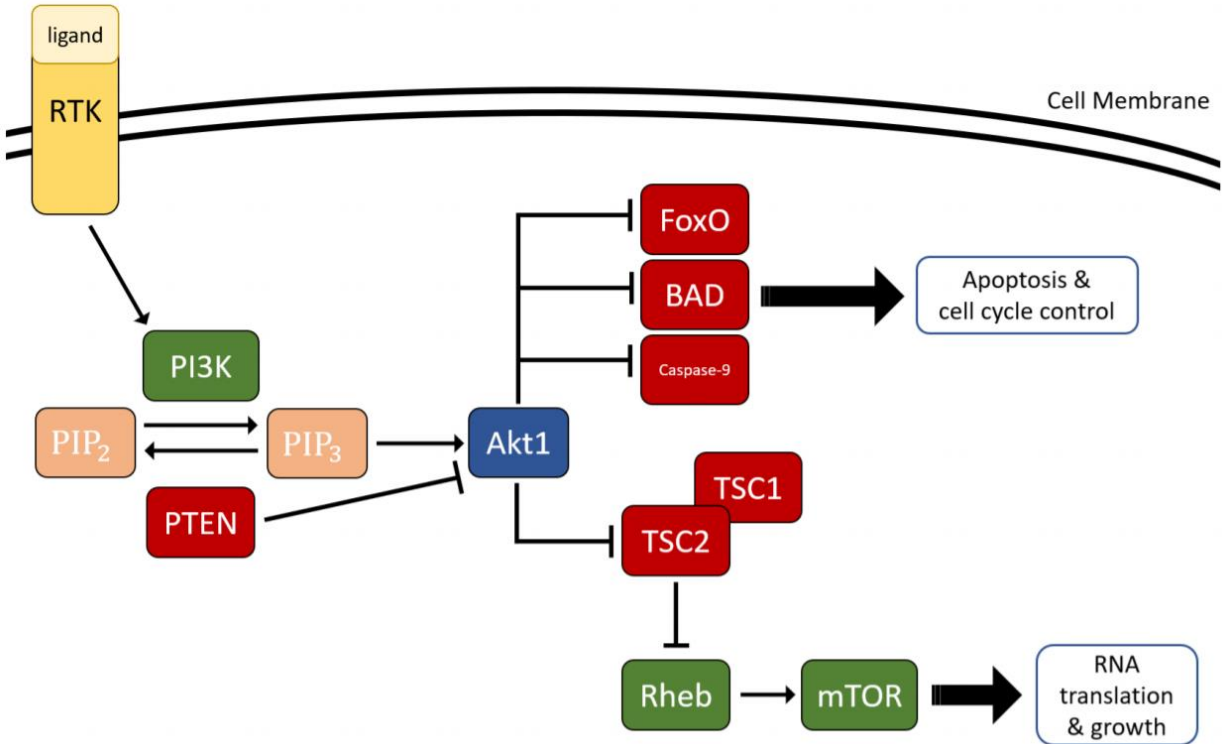
Figure 2: The PI3K pathway is a complex signaling pathway that includes PI3K, PTEN, and Akt1, a serine-threonine kinase. PI3K phosphorylation of PIP_2 to form PIP_3 is reversed by PTEN, which acts as a negative regulator for Akt1 activity. Apoptosis signaling proteins such as the FoxO transcription factors, BAD, and caspase-9 are all phosphorylated and inhibited by Akt1. In addition, Akt1 indirectly activates mTOR by inhibiting TSC2, enabling mTOR to further activate downstream targets to promote RNA translation and cellular growth (Dallarosa, 2018). The components of the PI3K pathway are often targeted for anti-cancer therapies.

Akt1

Akt1 is part of the Akt subfamily of serine-threonine protein kinases and is involved in the PI3K-driven pathways that regulate cell survival, metabolism, and migration (Ackah, et al., 2005). In particular, Akt1 can inhibit forkhead transcription factors such as FoxO1, which further enhances cellular proliferation and survival, common indicators of cancerous behavior (Choi and Anderson, 2017). In humans, the Akt1-encoding gene was observed to have 20-fold amplification in a form of gastric adenocarcinoma, which strongly suggests that *AKT1* is an oncogene (Staal, 1987).

As a part of the PI3K signaling pathway, Akt1 activity is regulated by PIP_3 binding to its pleckstrin homology (PH) domain. This domain is around 100 to 120 amino acids in length and is located on the N-terminus of Akt1. Interestingly, the PH domain is present in 250 different human proteins with differing amino acid sequences, yet the three-dimensional structure is highly conserved (Mahadevan, et al., 2008). However, only around 40 PH domain proteins have a high affinity for phosphoinositides, and these are the proteins that are crucial components of

the signaling pathways that promote cellular survival. PIP$_3$ recruits the PH domain to the cell membrane, and threonine-308 on the protein's activation loop and serine-473 on the C-terminus are phosphorylated (Altiok, et al., 1999) by PDK1. Akt1 activates other downstream proteins to suppress apoptosis signals (Mahadevan, et al., 2008). Specific inhibitors of the catalytic site of Akt1 have been developed for treating cancer, however, applications of these drugs have been hindered by toxicity (Mahadevan, et al., 2008).

EGFR
EGFR is a transmembrane protein that binds to ligands such as growth factors in the extracellular domain (Berg, 2012). Once bound, a ligand activates the phosphorylation of EGFR's own tyrosine residues and sends signals to both the MAPK pathway and the PI3K pathway. EGFR is considered an oncogene as its overexpression has been detected in 60-80% of colorectal cancer cells (Berg, 2012). In addition, in one-third of glioblastomas, the extracellular portion of EGFR has been truncated, leading to the protein signaling without ligand binding (Weinberg, 2014, p. 124). Tyrosine kinase inhibitors have been used to target EGFR, but this has produced dissatisfying results in cases of colorectal cancer (Berg, 2012). This development has led researchers to look further downstream along EGFR signaling pathways for more effective treatments.

EGFR undergoes dimerization and structural rearrangement when a ligand binds to the receptor. Tyrosine residues in the cytoplasmic portion of the receptor are auto-phosphorylated and this leads to further signaling along the MAPK, PI3K, and PLC pathways. EGFR signaling is regulated through internalization. Clathrin-mediated endocytosis occurs if the receptor is to be recycled rather than degraded in lysosomes (Sigismund, et al., 2017). Non-clathrin mediated endocytosis is considered to be a mechanism of protecting cells against excessive signaling.

Cancer Implications of IQGAP1, Akt1, and EGFR
Cancer cells have abnormally high growth, oddly shaped nuclei and morphology, and poor differentiation. Many proteins involved with maintaining these characteristics and homeostasis in non-cancerous cells can face some sort of irregularity, through mutations, deregulation, inactivation, or overexpression, resulting in tumorigenesis. Due to its ubiquitous presence, IQGAP1 has been detected in a broad range of human cancers, oftentimes overexpressed (Dallarosa, 2018). In addition, IQGAP1 binds to several proteins involved in the formation of tumors, including beta-catenin, Src, Cdc42, and Rac1 (White, et al., 2009). Under normal conditions, IQGAP1 and the tumor suppressor E-cadherin are located in the boundaries between epithelial cells. However, in gastric cancers, IQGAP1 disrupts the function of E-cadherin by moving from the cytoplasm to the cell periphery as the cancer cells become less differentiated (White, et al., 2009).

In addition, Akt1 is linked to cancer development. Under normal conditions, Akt1 promotes differentiation, growth, proliferation, and energy production for standard cellular functions and survival. However, overexpression and excessive activation of Akt1 enables it to continue these

processes uncontrollably. This results in transformed cells that can ignore apoptosis, grow irregularly and rapidly, and disregard cell cycle checkpoints. However, many kinase inhibitors for PI3K, Akt1, and mTOR have been developed as anti-cancer drugs and have even reached clinical development (Polivka, Jr. and Janku, 2013).

In brain, lung, and ovarian cancers, EGFR endocytosis is reduced, and this results in increased signaling. In addition, EGFR supports increased anaerobic glycolysis in the presence of oxygen, a phenomenon known as the Warburg Effect and commonly seen in cancer cells (Sigismund, et al., 2017). EGFR, when stimulated by EGF, regulates the expression of hexokinase and pyruvate kinase M2, two essential enzymes in the glycolysis process. The high levels of the glycolysis product, lactate, can inhibit immune responses from T-cells, further supporting cancer cell persistence. Furthermore, oncogenic mutations of EGFR in lung adenocarcinoma cells lead to excess activation of the PI3K pathway, which in turn leads to both stabilization of the glucose transporter GLUT1 and inhibition of TXNIP by Akt proteins (Sigismund, et al., 2017).

Research Aims
As shown in Figure 1, IQGAP1 has separate domains to bind to both Akt1 and EGFR. However, it is not confirmed that the respective bindings occur at the same time and in the same location of the cell. Understanding the spatial and temporal scaffolding of IQGAP1 with EGFR and Akt1 could have potential applications for anti-cancer therapies. Inhibiting IQGAP1 could prevent the scaffolding of EGFR and Akt1. If these proteins have oncogenic mutations, this could be successful in blocking irregular PI3K pathway signaling. Furthermore, extracellular signaling is the trigger for IQGAP1 scaffolding and for the PI3K pathway, and this step in the signaling cascade represents a potential point of therapeutic intervention as well.

The primary goal of this study was to determine the scaffolding effect of IQGAP1 on Akt1 and EGFR and observe protein interactions in human cancer cells. The binding of IQGAP1 and Akt1 was observed using Fluorescence Lifetime Imaging Microscopy (FLIM) following transfection of HeLa cells with IQGAP1-eGFP and Akt1-mCherry. Binding interactions between EGFR and Akt1 were observed using EGFR-eGFP and Akt1-mCherry. FLIM was utilized to detect and quantify any Förster Resonance Energy Transfer (FRET) between the two proteins in the presence of IQGAP1 and in response to EGF stimulation. Additionally, the interactions between EGFR and the PH domain of Akt1, which is an indicator of PIP_3, was observed in a similar manner. HeLa cells were grown and transfected with EGFR-eGFP and PH-Akt1-mCherry and then analyzed with FLIM. For this experiment, siRNA was used to knockdown IQGAP1 expression to observe the interactions of EGFR and PH-Akt1 in the absence of IQGAP1.

Methodology

DNA Plasmid Preparation
Plasmids that express fluorescently-tagged proteins were received as agar stabs from Addgene and stored at 4°C. A pipette tip was inserted into the stab and added to 200mL of sterile LB. The flask was shaken at 220rpm at 37°C for 24 hours. The resulting *E. coli* culture was centrifuged at 8,000rpm for 10 minutes and the pellet was collected. Plasmid DNA was isolated from the *E. coli* pellet by following the manufacturer's instructions of a QIAGEN DNA mini-prep purification kit. DNA concentrations were determined using a Nanodrop spectrophotometer with the QIAGEN kit elution buffer used as a blank. DNA samples were stored at 4°C.

Cell Culture and Maintenance
All experiments in this study were carried out in the HeLa cell line. Culture media was composed of Dulbecco's Modified Eagle Medium (DMEM), and 5% Fetal Bovine Serum (FBS). Cell cultures were grown at 37°C and 5.0% CO_2. Cells were grown in 100mm dishes with 10mL of media, which was suctioned out and replaced with fresh media every 48 hours. When a dish reached approximately 90% confluency, the culture was split following a wash with 5mL of Dulbecco's phosphate-buffered saline (DPBS) and a five-minute incubation period with 1mL trypsin.

Transfection
HeLa cell transfection was completed by following the Thermo Fisher Lipofectamine 3000 protocol. One microcentrifuge tube containing 100μL of OPTI-MEM media, 5μg of DNA, and 5μL of P3000 Reagent was combined with another microcentrifuge tube containing 100μL of OPTI-MEM media and 5μL of Lipofectamine. For the IQGAP1 knockdown experiments, 5μL of IQGAP1 siRNA for the P3000 reagent. After 15 minutes of incubation in the hood at room temperature (23°C), the DNA-lipid complex was added to a 2-cm dish containing 2mL of antibiotic-free FBS media. Dishes were left in the incubator at 37.0°C for 48 hours before analysis and visualization.

Confocal Imaging
Cell media was removed from the dishes via vacuum suction and then the cells were washed with Tris buffered saline (TBS) buffer. The media was replaced with 2mL of serum-free DMEM. For experiments that tested response to external stimuli, 100ng/mL of EGF was added. Images of the cells were obtained using a Zeiss LSM 510 confocal microscope with a 40X water objective. Zeiss and ImageJ software packages were used to analyze the cell images.

Fluorescence Lifetime Imaging Microscopy (FLIM)
FLIM is a method of observing and calculating FRET between tagged proteins in live cells. Physical protein contact is essential for a donor fluorophore to transfer its energy to an acceptor fluorophore due to the inverse relationship between efficiency and distance. When the fluorescence energy of the donor is transferred to an acceptor, the fluorescence lifetime of the donor is reduced. This change in fluorescence lifetime can be monitored and quantified with

FLIM. In this study, enhanced green fluorescent protein (eGFP) was used as the donor fluorophore while mCherry was used as the acceptor fluorophore.
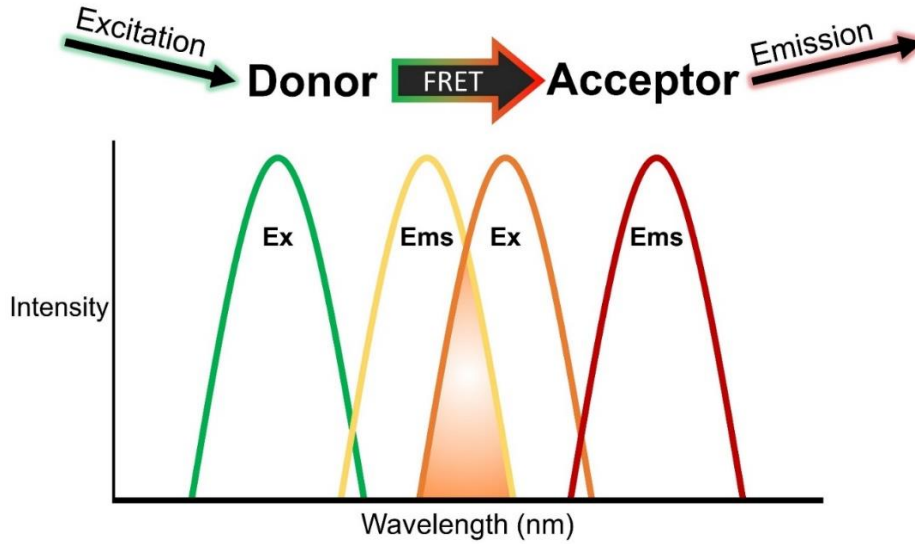

Figure 3: FLIM is a technique used for the observation and quantification of FRET between two fluorophores. Adapted from Giquel, B. 2014, Nov 5. Tips for Using FRET in Your Experiments. Retrieved from https://blog.addgene.org/tips-for-using-fret-in-your-experiments

Following the 48-hour incubation period after transfection, culture media was removed via vacuum suction and replaced with serum-free DMEM for imaging. For experiments that tested responses to external stimuli, 100ng/mL of EGF was added and was incubated for at least an hour before images were taken. Cells were imaged with a Nikon inverted confocal microscope using a 60X water objective and a two-photon MaiTai laser. Multiple (5-6) images were taken for each dish, each focusing on a different cell that displayed transfection efficiency for both plasmids. Images were analyzed with ISS VistaVision and ImageJ software packages. Each pixel of a FLIM image has a fluorescence phase shift and modulation at the emission frequency that can be graphically represented as a vector in a phasor plot, which are shown in Figures 7 and 9. The G and S values are calculated by the following equations (Povrozin, et al., 2013):

$$G_{h,k}(\omega) = \frac{\int_0^\infty I_{h,k}(t)\cos\omega t\, dt}{\int_0^\infty I_{h,k}(t)dt} = m_{h,k}\cos(\varphi_{h,k})$$

$$S_{h,k}(\omega) = \frac{\int_0^\infty I_{h,k}(t)\sin\omega t\, dt}{\int_0^\infty I_{h,k}(t)dt} = m_{h,k}\sin(\varphi_{h,k})$$

Where $m_{h,k}$ represents the demodulation ratio, $\varphi_{h,k}$ represents the phase shift, ω represents modulation frequency, and (h, k) represents the horizontal and vertical location of the pixel in a FLIM image. A right-ward shift of the modulation vector, $\vec{m}$, indicates that FRET is occurring between the donor and the acceptor and thus, physical proximity of the tagged proteins (Povrozin, et al., 2013).

Results

EGFR-eGFP is internalized in presence of excess EGF

For 180 minutes, a series of images was taken of a single cell transfected with EGFR-eGFP and stimulated with 1µL of 1ng/mL EGF using a Zeiss LSM 510 confocal microscope. Key time points are shown below:
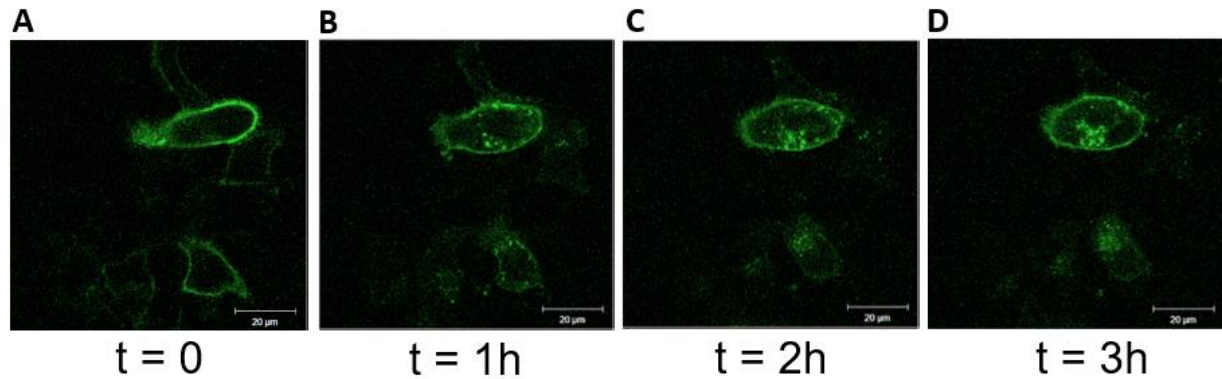

Figure 4A-D: Images of a EGFR-eGFP transfected HeLa cell taken as a time-lapse series over the span of 180 minutes. From left to right, small fluorescent green particles appear on the plasma membrane, and then in the cytoplasm. It has been previously mentioned that EGFR is internalized by cells for recycling and degradation after binding to a cognate ligand.

This image series strongly suggests that EGFR-eGFP is internalized and sent to lysosomes for degradation in the same way as untagged EGFR. Therefore, the EGFR-eGFP used in later experiments will behave in a similar way to untagged EGFR. This experiment primarily served as validation for this specific fluorescent-tagged plasmid that would be used in later experiments.

IQGAP1 and Akt1 strongly interact at the plasma membrane in an EGF independent manner & EGFR and Akt1 weakly interact at the plasma membrane in the presence of IQGAP1

The interaction between IQGAP and Akt1 at the plasma membrane allows IQGAP1 to scaffold elements of the PI3K signaling pathway and to disrupt interactions with MAPK pathway proteins.

EGFR and Akt1 are both upstream regulators of the PI3K pathway. EGFR responds to external signals and relays the message to PI3K. Then, PI3K generates PIP_3 that recruits Akt1 to the plasma membrane. Essentially, EGFR and Akt1 are a part of the same signaling cascade, however, there is one protein that is in between the two in terms of signaling. Here, it is discovered that this step may be a temporal or spatial obstacle for FRET to occur between EGFR and Akt1.
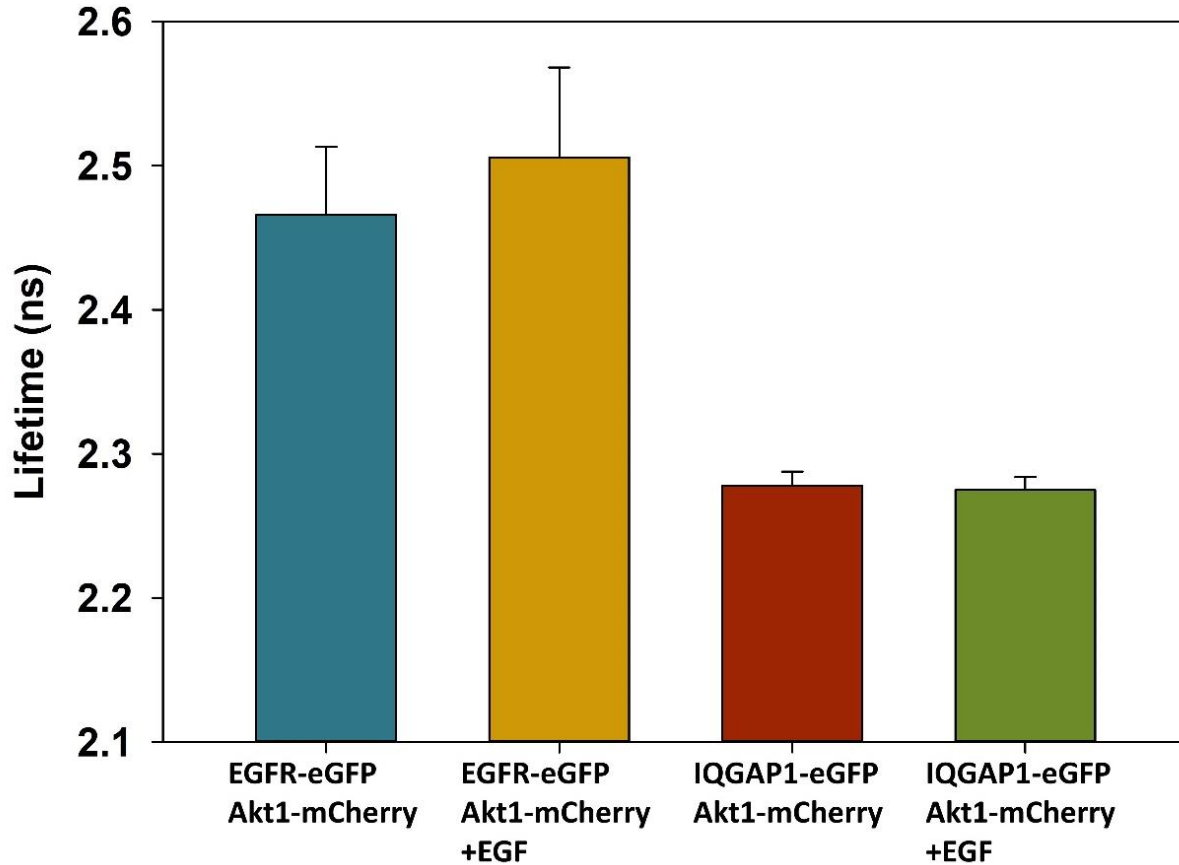
Figure 5: FLIM/FRET analysis of EGFR-eGFP/Akt1-mCherry and IQGAP1-eGFP/Akt1-mCherry Transfected HeLa Cells: FLIM/FRET measurements of EGFR-eGFP and Akt1-mCherry in the presence of IQGAP1 show a slight reduction of fluorescence lifetime compared to the EGFR control (Figure 8). EGF stimulation does not appear to impact the interaction between EGFR and Akt1. These results suggest that while IQGAP1 is binding to both proteins, they are not in close enough proximity to result in FRET. FLIM/FRET measurements of IQGAP1-eGFP and Akt1-mCherry show an increase in proximity compared to EGFR and Akt1, as indicated by the decrease in fluorescence lifetime. The decrease in fluorescence lifetime confirms that IQGAP1 and Akt1 are in very close proximity and FRET occurs between the two. EGF stimulation does not appear to have any effect on the interaction between IQGAP1 and Akt1. Since EGF is a stimulant that binds to EGFR, it follows that this ligand would not affect this interaction (n ≥ 4).
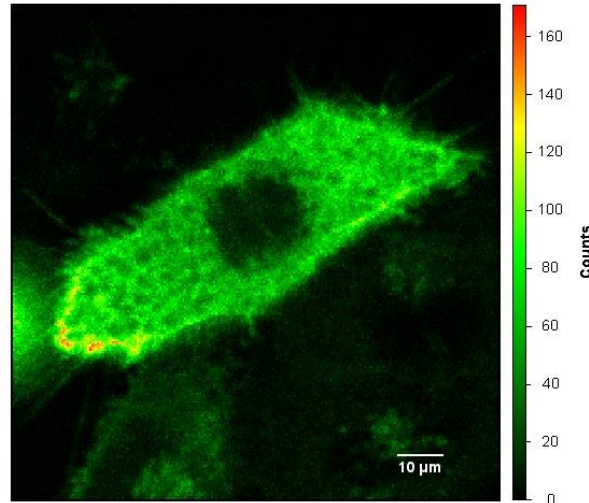
Figure 6: FLIM Image of IQGAP1-eGFP and Akt1-mCherry Transfected HeLa Cell. This image taken of a HeLa cell expressing IQGAP1-eGFP and Akt1-mCherry shows that a high count of fluorescent photons is found at the plasma membrane on the leftward edge of the cell. This indicates that IQGAP1 and Akt1 are in close proximity at the plasma membrane, which is where Akt1 is recruited to by PIP_3 and where IQGAP1 acts as a scaffold. A high count of fluorescent pixels, shown by a red, orange, or yellow color, are detected at a growth front of this cell. This suggests that Akt1 and IQGAP1 are directing growth and motility processes at this location.
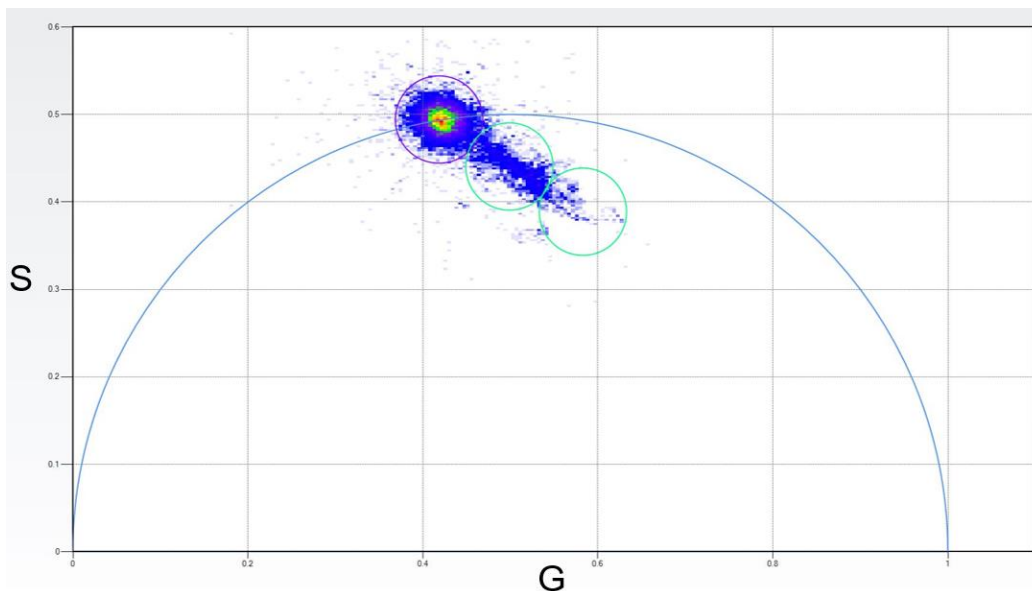

Figure 7: Phasor plot of IQGAP1-eGFP/Akt1-mCherry: As a result of the reduction in fluorescence lifetime of IQGAP1-eGFP, the phase shift and modulation values move inside the Gaussian distribution curve. Data points inside the purple circle represent longer (non-FRET) lifetimes of IQGAP1 while values inside the teal circles represent shorter FRET lifetimes. These results suggest that IQGAP1 and Akt1 are in close proximity and experience FRET. FLIM data and phasor plot analysis was performed using the ISS VistaVision software package.
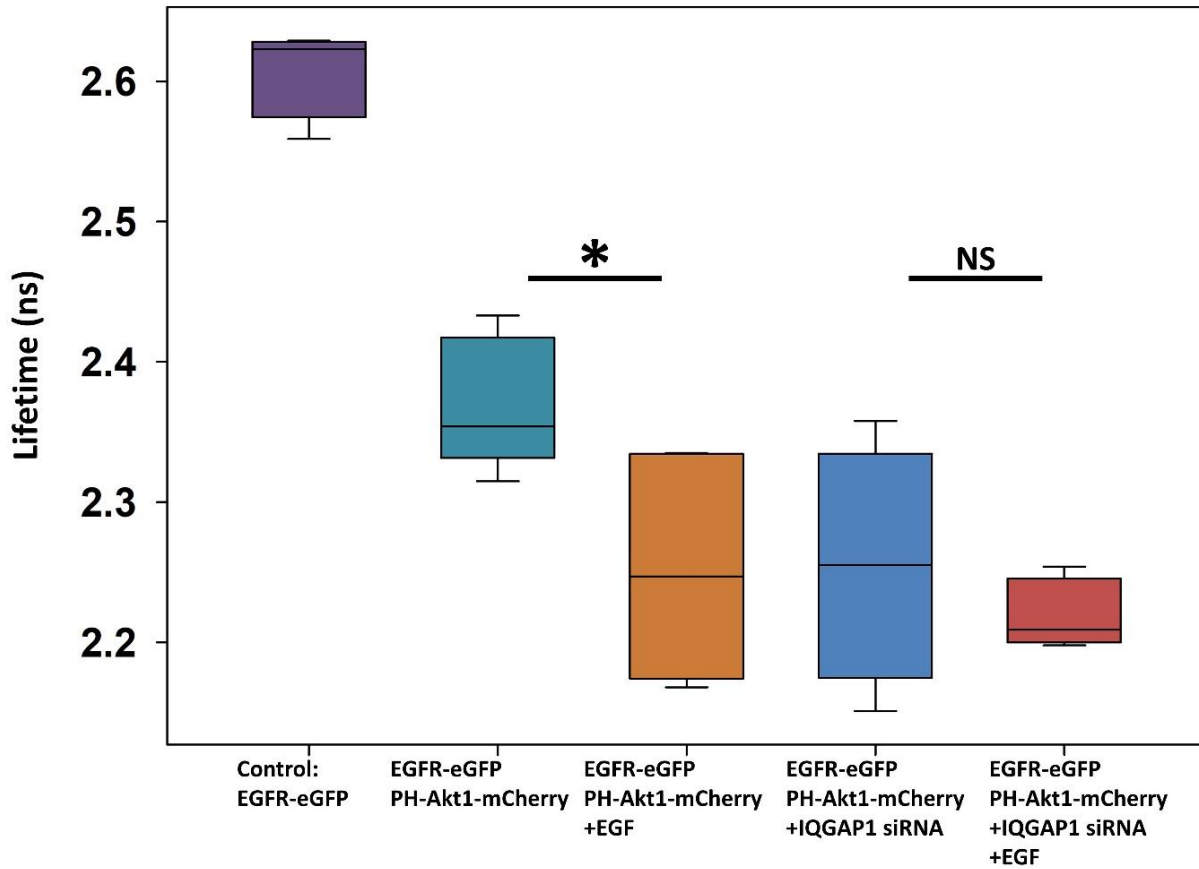
Figure 8: FLIM/FRET Analysis of EGFR-eGFP/PH-Akt1-mCherry transfected HeLa Cells: FLIM/FRET measurements of EGFR-eGFP and PH-Akt1-mCherry, a sensor for PIP$_3$, in the presence and absence of IQGAP1. FLIM analysis of EGFR and the PH domain of Akt1 show a significant reduction in fluorescence lifetime compared to the EGFR control. EGF stimulation increases FRET, as indicated by the lower fluorescence lifetimes observed between the PH domain of Akt1 and EGFR. IQGAP1 knockdown eliminated the effect of the EGF stimulation on the interaction of the PH domain of Akt1 and EGFR. This suggests that IQGAP1 is necessary for bringing PIP$_3$ and EGFR close together, and the physical distance between the two decreases further upon EGF stimulation. All test transfections were statistically significant ($p \leq 0.01$) from the control. ($n \geq 4$, * = $p \leq 0.05$, NS = $p \geq 0.05$).
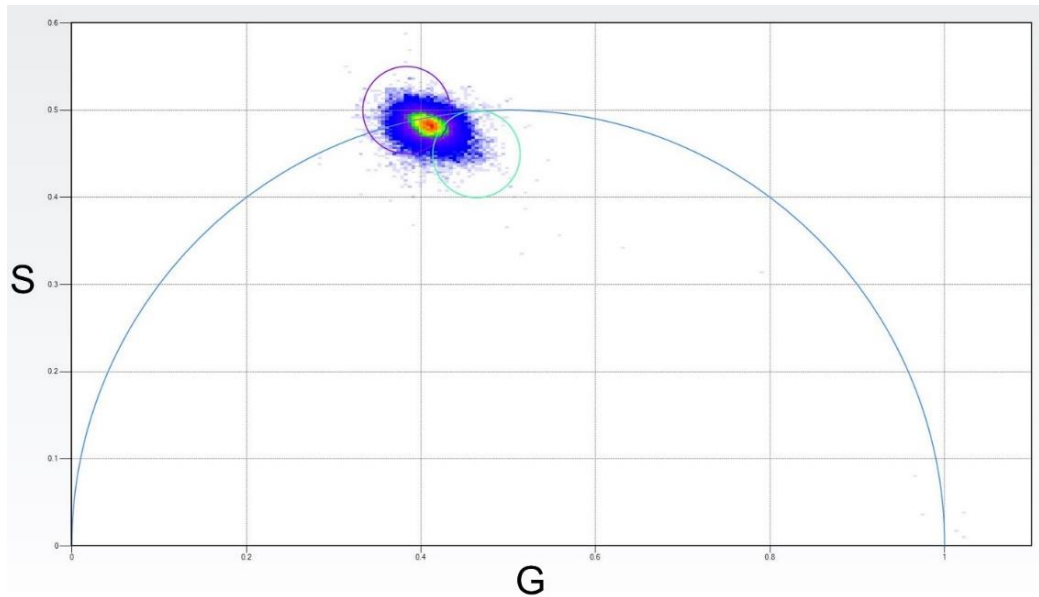
Figure 9: Phasor plot of EGFR-eGFP/PH-Akt1-mCherry with EGF stimulation: When co-expressed with PH-Akt1-mCherry, EGFR-eGFP fluorescence lifetime values move inside the Gaussian arc, which is a result of the reduction in fluorescence lifetime caused by FRET between the closely localized proteins. Data points inside the purple circle represent longer (non-FRET) lifetimes of EGFR while values inside the teal circle represent shorter FRET lifetimes. These results suggest that EGFR and the PH domain of Akt1 are in close proximity and experience FRET. FLIM data and phasor plot analysis was performed using the ISS VistaVision software package.
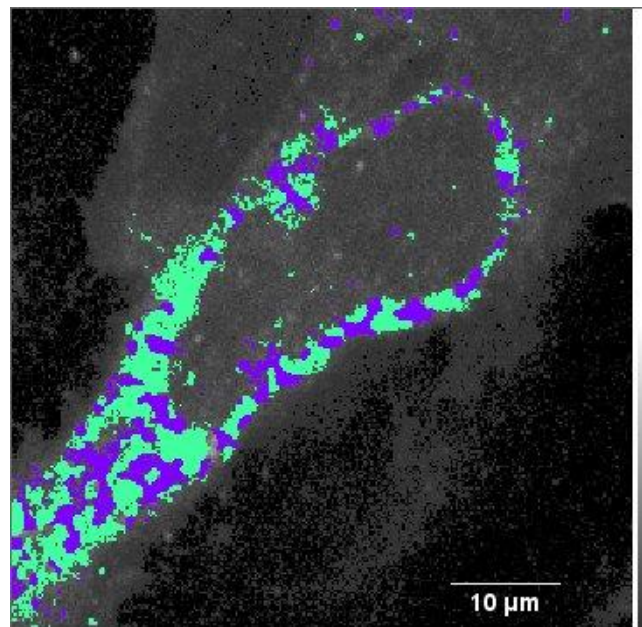

Figure 10: EGFR-eGFP/PH-Akt1-mCherry Transfected HeLa cell image overlaid with phasor plot. As previously mentioned in Figure 9, purple regions represent longer, non-FRET lifetimes of EGFR-eGFP while the teal regions represent shortened fluorescence lifetimes. These results indicate that EGFR and PH-Akt1 are in close proximity, and thus enable FRET to occur between the two proteins.

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Discussion and Conclusion

In this study, we discovered that EGFR and the PH domain of Akt1 interact in the presence of IQGAP1 and this interaction is enhanced with EGF stimulation. EGFR and Akt1 weakly interact at the plasma membrane, and this interaction does not appear to be affected by EGF stimulation. Finally, it was confirmed that IQGAP1 and Akt1 strongly interact at the plasma membrane in an EGF-independent manner. These results suggest that IQGAP1 scaffolds EGFR, Akt1, and PIP_3 to regulate upstream PI3K signaling pathway components in response to the external stimulus EGF.

Previous studies in the Gericke lab group have confirmed that IQGAP1 plays a role in scaffolding PIPKIγ with talin and Cdc42, and with PI3K. In addition, IQGAP1 itself associates with PIP_2, PIP_3, PIPKIγ, β-actin, and talin. IQGAP1 has also been shown to form clusters in response to stimulation with EGF. These results suggest that IQGAP1 is able to scaffold cytoskeletal and phosphoinositides together for cell motility regulation and cytoskeletal reorganization. This study allows for the addition of Akt1 and EGFR to the proposed model of the IQGAP1 scaffold at the plasma membrane (Figure 11). This multiprotein complex enables IQGAP1 to mediate signaling from both cytoskeletal regulatory proteins and proteins involved in phosphoinositide signaling pathways. Cellular functions such as migration, focal adhesions, and cytoskeletal reorganization can be regulated by multiprotein complexes generated by IQGAP1 scaffolding.

Future studies could examine the role of IQGAP1 in scaffolding EGFR and Akt1 specifically. Although it was found that EGFR and Akt1 do not appear to be in close proximity in the presence of IQGAP1 in this study, further experiments with IQGAP1 knockdown with siRNA is necessary to confirm those results.

Additional future studies could be geared towards further investigation of IQGAP1 as a scaffolding protein. Since IQGAP1 is known to bind to a large number of different proteins, there are many additional potential binding interactions that can be examined. For example, the FLIM completed for this study could be repeated with PDK1 or MAPK1 in the place of Akt1. The MAPK/ERK pathway is comparable to the PI3K/AKT pathway in terms of controlling cell survival, proliferation, and metabolism. Interestingly, these pathways are negative regulators of each other, and therefore each pathway can be inhibited by the other pathway's component proteins. For example, Akt1 can phosphorylate and inhibit Raf, which is an upstream activator of ERK (Mendoza et al., 2012). On the other hand, ERK can inhibit GAB, which is an effector protein for the p85 subunit of PI3K. Given that these two pathways are antagonistic and analogous could indicate that there are downstream kinases in the MAPK/ERK pathway that are candidates for binding with IQGAP1 to regulate cellular proliferation in a similar way as Akt1.

Lastly, future studies could determine if there are other transmembrane receptors that are involved in IQGAP1 scaffolding. For example, Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) binds to IQGAP1 directly to promote blood vessel generation (Dallarosa, 2018). Cancer cells often depend on angiogenesis to support a growing tumor with oxygen, nutrients, and remove metabolic waste. VEGF is excessively secreted by cancer cells to bind to their own

VEGF receptors through autocrine signaling. In a study of a murine cancer model, angiogenesis was significantly enhanced with IQGAP1 overexpression (Hedman, et al., 2015). Furthermore, mice with IQGAP1 absent had reduced recovery and blood circulation following injury and ischemia, respectively. This study suggests that IQGAP1 is involved in VEGF-initiated angiogenesis (Hedman, et al., 2015). IQGAP1 scaffolding of VEGFR2 could regulate blood vessel formation, maintenance, and repair.

In conclusion, IQGAP1 plays an important role in the cell as a scaffolding protein for multiple pathways and processes. IQGAP1 is a scaffolding protein that is able to regulate key cytoskeletal and phosphoinositide signaling processes through the generation of a multiprotein binding complex. EGFR, Akt1, and PIP_3 are key elements to this scaffold and enable IQGAP1 to specifically regulate the PI3K/Akt/mTOR pathway.
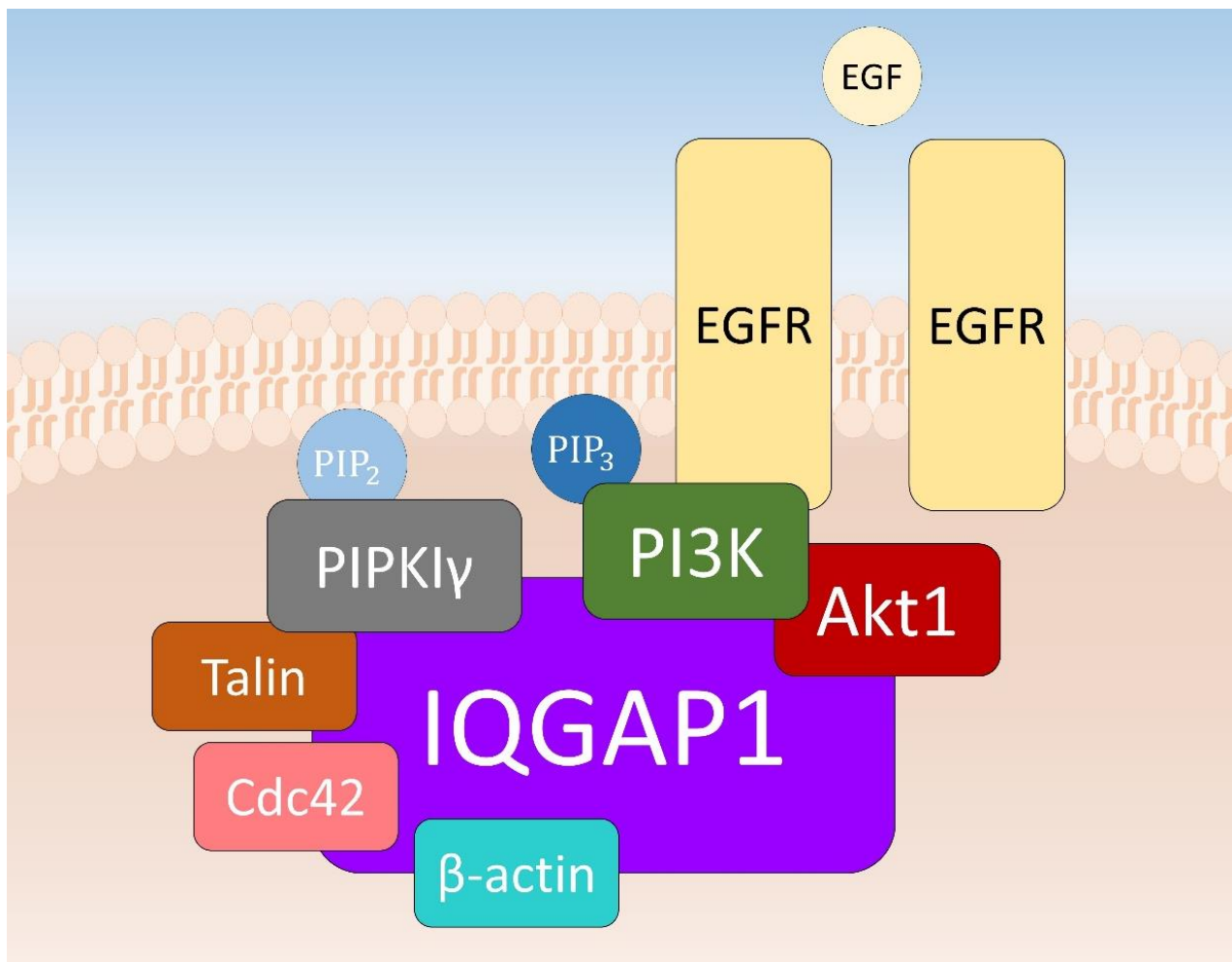

Figure 11: The proposed model of the IQGAP1 scaffold at the plasma membrane. This multiprotein complex scaffolded by IQGAP1 includes cytoskeletal regulatory proteins, phosphoinositides, PIPKIγ, and PI3K pathway proteins. Cellular functions such as migration, focal adhesions, and cytoskeletal reorganization can be regulated by the scaffolded complex.

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