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Article *in* Advances in Biological Regulation · September 2017

DOI: 10.1016/j.jbior.2017.09.001

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Phosphoinositide 5-phosphatase activities control cell motility in glioblastoma: Two phosphoinositides PI(4,5)P2 and PI(3,4)P2 are involved

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ARTICLE INFO

Article history:
Received 25 August 2017
Received in revised form 1 September 2017
Accepted 1 September 2017
Available online xxx

Keywords:
Phosphoinositides
PI(4,5)P2
SHIP1/2
Cell migration
Signal transduction

ABSTRACT

Inositol polyphosphate 5-phosphatases or phosphoinositide 5-phosphatases (PI 5-phosphatases) are enzymes that can act on soluble inositol phosphates and/or phosphoinositides (PIs). Several PI 5-phosphatases have been linked to human genetic diseases, in particular the Lowe protein or OCRL which is mutated in the Lowe syndrome. There are 10 different members of this family and 9 of them can use PIs as substrate. One of these substrates, PI(3,4,5)P3 binds to specific PH domains and recruits as effectors specific proteins to signaling complexes. Protein kinase B is one target protein and activation of the kinase will have a major impact on cell proliferation, survival and cell metabolism. Two other PIs, PI(4,5)P2 and PI(3,4)P2, are produced or used as substrates of PI 5-phosphatases (OCRL, INPP5B, SHIP1/2, SYNJ1/2, INPP5K, INPP5J, INPP5E). The inositol lipids may influence many aspects of cytoskeletal organization, lamellipodia formation and F-actin polymerization. PI 5-phosphatases have been reported to control cell migration, adhesion, polarity and cell invasion particularly in cancer cells. In glioblastoma, reducing SHIP2 expression can positively or negatively affect the speed of cell migration depending on the glioblastoma cell type. The two PI 5-phosphatases SHIP2 or SKIP could be localized at the plasma membrane and can reduce either PI(3,4,5)P3 or PI(4,5)P2 abundance. In the glioblastoma 1321 N1 cells, SHIP2 controls plasma membrane PI(4,5)P2 thereby participating in the control of cell migration.

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http://dx.doi.org/10.1016/j.jbior.2017.09.001
2212-4926/

Please cite this article in press as: Ramos, A.R., et al., Phosphoinositide 5-phosphatase activities control cell motility in glioblastoma: Two phosphoinositides PI(4,5)P2 and PI(3,4)P2 are involved, Advances in Biological Regulation (2017), http://dx.doi.org/10.1016/j.jbior.2017.09.001

1. Introduction

Inositol polyphosphate 5-phosphatases or phosphoinositide 5-phosphatases (PI 5-phosphatases) are enzymes that can act on soluble inositol phosphates and/or phosphoinositides (PIs). They catalyze the dephosphorylation of the phosphate at 5 position of the inositol ring (Balla, 2013; Elong Edimo et al., 2012; Eramo and Mitchell, 2016). In human cells, there are ten different members of this family (Table 1). Except INPP5A (or type I I(1,4,5)P3 5-phosphatase) that can only use I(1,4,5)P3 and I(1,3,4,5)P4 as substrate (i.e. soluble inositol phosphates acting as second messengers of Ca^{2+} mobilization (De Smedt et al., 1997)), the other enzymes can take both inositol phosphates and PIs as substrate or allosteric modulator (Balla, 2013; Ong et al., 2007). It is generally accepted that only PIs are recognized as substrate in intact cells for OCRL, INPP5B, INPP5J, INPP5K, INPP5E, SYNJ1/2 and SHIP1/2 (Table 1). PI 5-phosphatases are therefore critical in the control of PI(3,4,5)P3, PI(4,5)P2, and PI(3,5)P2 intracellular content and related signaling properties. The importance of inositol and PI 5-phosphatase in many human genetic diseases is now well established. The PI 5-phosphatase OCRL was the first enzyme of this family identified as being mutated in the Lowe syndrome and Dent-2 disease (Attree et al., 1992). More recently, SYNJ1, INPP5K and SHIP2 have been found to be mutated in Parkinson disease (Drouet and Lesage, 2014), some congenital muscular dystrophy (Osborn et al., 2017) and opsismodysplasia (Below et al., 2013; Huber et al., 2013), respectively (Table 1). Moreover, the same enzymes, in particular SYNJ2, SHIP2 and INPP5J, have also been reported as tumor promoting and tumor suppressors in some cancers such as breast cancer, glioblastoma or squamous cell carcinoma (Erneux et al., 2016). For example, INPP5J is a tumor suppressor and SYNJ2 shows oncogenic activity in some breast cancer cells (Ben-Chetrit et al., 2015; Ooms et al., 2015a). SHIP2 is also oncogenic in breast cancer but a tumor suppressor in squamous cell carcinoma (Prasad et al., 2008; Yu et al., 2008). The reason for the discrepancies observed in different cancer models is not known. It could be linked to activity/specificity or non-catalytic effects of the different PI 5-phosphatases that also interact with a series of non-common protein interactors. This network of specific proteins is probably as important as the catalytic properties to understand function (Erneux et al., 2011).

There is a debate concerning whether PI(3,4)P2 contributes to protein kinase B and downstream effector activation together with PI(3,4,5)P3 and whether SHIP2 docking properties are important to modulate the tumor promoting response for example in breast cancer. Specific interactions between SHIP2 and Mena, an Ena/VASP-family actin regulatory protein, have been reported to suggest that SHIP2 regulation of invadopodia requires an intact proline rich sequence via a phosphatase independent mechanism (Rajadurai et al., 2016). Moreover, recently, novel second messenger functions of PI(3,4)P2 have been identified in the control of invadopodium maturation (Eddy et al., 2017), feedback control of PI(3,4,5)P3 generation in breast cancer cells (Reed and Shokat, 2017) and of basal mTORC1 activity in many different cells (Marat et al., 2017). Therefore, PI(3,4)P2 must be considered as a second messenger on its own (Li and Marshall, 2015). Its role could be particularly relevant in cancer cells where INPP4B is mutated or absent, a situation that frequently occurs in aggressive hormone receptor-negative basal-like breast carcinomas (Fedele et al., 2010).

PI(3,4)P2 also recruits lamellipodin (Ras association and PH domains 1), a protein that specifically recognizes PI(3,4)P2 as a ligand at the plasma membrane (Krause et al., 2004). The interaction and recruitment promotes lamellipodia formation and directional cell migration. In B cells, SHIP1 is particularly abundant and can produce PI(3,4)P2. Lamellipodin and PI(3,4)P2 are colocalized and PI(3,4)P2 binding to lamellipodin is found to mediate directional migration. Depletion of PI(3,4)P2 in primary chronic lymphocytic leukemia impairs cell migration (Li et al., 2016).

2. The influence of PI 5-phosphatases OCRL, INPP5J, SKIP, SHIP1 and SHIP2 on cell migration

PI 5-phosphatases have been reported to influence cell migration, adhesion and polarity. Lowe syndrome patient fibroblasts display OCRL-1 specific cell migration and spreading defects (Coon et al., 2009). These abnormalities were suppressed by expressing wild-type OCRL1 but not its catalytic mutant suggesting that PIs are important in the mechanism. PI(4,5)P2 levels are higher in patient fibroblasts relative to control (Zhang et al., 1998). It was therefore proposed that cells derived from Lowe patients would exhibit deficiencies in their ability to promote the PI(4,5)P2 turnover required to generate the leading edge (Coon et al., 2009).

Table 1
Human PI 5-phosphatases associated genetic diseases.

Protein	Gene name	Associated genetic disease
Type I inositol 1,4,5-trisphosphate 5-phosphatase	*INPP5A*	Not reported
Inositol polyphosphate 5-phosphatase K (SKIP)	*INPP5K*	Congenital muscular dystrophy syndrome (Osborn et al., 2017)
SH2 domain-containing inositol 5-phosphatase 1 (SHIP1)	*INPP5D*	Crohn's disease (Somasundaram et al., 2017)
SH2 domain-containing inositol 5-phosphatase 2 (SHIP2)	*INPPL1*	Opsismodysplasia (Below et al., 2013; Huber et al., 2013)
Phosphatidylinositol 4,5-bisphosphate 5-phosphatase A	*INPP5J*	Not reported
Inositol polyphosphate 5-phosphatase OCRL-1	*OCRL*	Lowe syndrome (Attree et al., 1992); Dent-2 disease (Bokenkamp et al., 2009)
Type II inositol 1,4,5-trisphosphate 5-phosphatase	*INPP5B*	Not reported
Synaptojanin-1	*SYNJ1*	Parkinson (Drouet and Lesage, 2014)
Synaptojanin-2	*SYNJ2*	Not reported
72 kDa inositol polyphosphate 5-phosphatase	*INPP5E*	Ciliopathy Joubert and MORM syndromes (Bielas et al., 2009; Jacoby et al., 2009)

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Skeletal muscle and kidney inositol phosphatase (SKIP) is encoded by the *INPP5K* gene (Ijuin et al., 2000). This enzyme has been mainly studied in the context of insulin signaling where it appears to use PI(3,4,5)P3 as substrate, to inhibit insulin signaling and subsequently glucose incorporation (Ijuin and Takenawa, 2003). In cancer cells, SKIP shows both increased and decreased expression in human PTEN-deficient glioblastomas (Davies et al., 2014). This is in agreement with large-scale microarrays data that reported increased or decreased *SKIP* mRNA or DNA levels in glioblastoma relative to normal tissue (Bredel et al., 2005; Liang et al., 2005; Murat et al., 2008). The effect of SKIP knockdown or overexpression was therefore investigated in the PTEN-null U-87MG glioblastoma cell line (Davies et al., 2014). In a context of integrin stimulation, SKIP depletion inhibited cell migration possibly via increased PI(4,5)P2 abundance. Whether PI(3,4,5)P3 and PI(3,4)P2 are modified in this mechanism is not yet clear.

In breast cancer, INPP5J (or PIPP) depletion reduces cell migration in a mechanism that depends specifically on AKT1 (Ooms et al., 2015b). SHIP1 is also important in the control of cell migration and chemotaxis: Constitutively active SHIP1 in T cell was reported to suppress chemotaxis (Wain et al., 2005). The integrin adhesome network (see http://www.adhesome.org) include SHIP1 and SHIP2. This supports a role of SHIP2 in many different cytoskeletal associated mechanisms such as cell adhesion (Prasad et al., 2001), migration (Kato et al., 2012; Venkatareddy et al., 2011) and cell polarity (Awad et al., 2013).

3. PI(4,5)P2 control cell polarity, cytoskeletal reorganization and cell migration

The link between PI(4,5)P2 synthesis and degradation is associated to multiple mechanisms that will lead to efficient cell polarity and migration. PI(4,5)P2 directly regulates a very large number of proteins that participate at many steps of the cytoskeletal organization (Thapa et al., 2016). PI(4,5)P2 interaction with myosin, dynamin, gelsolin, neural Wiskott-Aldrich syndrome protein (N-WASP) has been reported. Regulation of the actin-nucleating activity by PI(4,5)P2 via WASP and Rho GTPases is another mechanism that potentiates cell migration. PI(4,5)P2 is also connected to focal adhesions: PIPKIγ, which produces PI(4,5)P2, targets and regulate focal adhesions (Ling et al., 2002). The targeting occurs by the association with talin. The PI(4,5)P2 generating enzyme or PIPKIγ interacts with the cytoskeletal regulator and scaffold IQGAP1 and both proteins function together in synergy in the regulation of directional cell migration (Choi et al., 2013). Finally, the PI 5-phosphatase OCRL is important to establish cell polarity in the control of PI(4,5)P2 turnover a mechanism which is lost in Lowe patient cells (Grieve et al., 2011). In summary, PI(4,5)P2 which is a substrate for many PI 5-phosphatases i.e. OCRL, INPP4B, SKIP, SHIP1/2, influences F-actin polymerization at many steps.

4. Two major PIs control cell migration in glioblastoma being cell type dependent

Glioblastoma is one of the most challenging form of cancer to treat (Sami and Karsy, 2013). Mutation of PTEN and subsequent upregulation of PKB/mTOR are commonly seen in primary glioblastoma (Brennan et al., 2013). In this type of cancer, PI 3-kinase mutations were mutually exclusive of PTEN mutations/deletions with 59% of glioblastoma showing one or the other (TCGA glioblastoma Analysis Working Group).

Moreover, proliferation and self-renewal of glioblastoma cancer stem cells are targetable by novel mTORC1 and mTORC2 inhibitors (Jhanwar-Uniyal et al., 2017). SHIP2 expression is very much variable between different primary glioblastoma being either highly expressed or very low and in some cases even undetectable by Western blotting (William's Elong Edimo and Pierre Robe, GIGA, Université de Liège, unpublished data). In the PTEN-null glioblastoma cell model 1321 N1 cells, it was observed that SHIP2 controls PI(3,4,5)P3 levels and PKB activity (Elong Edimo et al., 2011), as also reported in other glioblastoma cells such as U-87MG (Taylor et al., 2000). This has an impact on cell proliferation and/or apoptosis suggesting a tumor suppressor function in this model (Elong Edimo et al., 2014). Although PI(3,4,5)P3 is the best substrate of SHIP2 as compared to other PIs in a phosphatase assay, PI(4,5)P2 is also a substrate in such type of assays (Giuriato et al., 2002). In 1321 N1 cells, SHIP2 is not acting *only* as a PI(3,4,5)P3 5-phosphatase. It can also act as a PI(4,5)P2 5-phosphatase, thereby controlling the ratio between PI(4,5)P2 and PI4P, focal adhesion turnover and cell migration (Elong Edimo et al., 2016a). PI(4,5)P2 is upregulated in SHIP2 depleted N1 cells as compared to control cells and the speed of cell migration is increased in N1shSHIP2 cells as compared to control cells. Cells transfected with GFP-PH/Btk (a biosensor of PI(3,4,5)P3) and GFP-PH/PLCδ1 (a biosensor for PI(4,5)P2) have been compared in SHIP2 depleted cells. Live cell imaging of the two GFP constructs shows that GFP-PH/Btk decreases cell migration reducing cell velocity by only 17% as compared to GFP alone whereas GFP-PH/PLCδ1 reduces velocity by 65%. This suggested that PI(4,5)P2 plays a major role in the control of cell migration in that model (Elong Edimo et al., 2016a). In 1321 N1 cells, cell migration was not inhibited by two PI 3-kinase inhibitors LY-294002, and wortmannin as well as by the PKB inhibitor Akti. PI 3-kinase inhibitors do however inhibit cell migration in a different glioblastoma cell line LN229 cells. In LN229 cells, depletion of SHIP2 inhibited cell migration. So the negative control of SHIP2 on cell migration in 1321 N1 cells is very much cell type specific and varies between different glioblastoma cells. As PI(3,4)P2 is interacting with lamellipodin and has a major role in lamellipodia formation, we suggested that cell migration in glioblastoma could be controlled by two PIs at least: PI(3,4)P2 which facilitates lamellipodia formation and PI(4,5)P2 acting on focal adhesions (Elong Edimo et al., 2016b; Elong Edimo et al., 2016a).

The influence of SHIP2 on PI(4,5)P2 was also reported in non-cancer cells: in mice, SHIP2 controls renal brush border ultrastructure and function by regulating the activation of the ERM proteins. In this model, PI(4,5)P2 was increased and PI4P decreased when SHIP2 was inactivated (Sayyed et al., 2017). In another study, in MDCK cells, SHIP2 may affect PI(4,5)P2 levels to control cell division and PI(3,4)P2/PI(3,4,5)P3 to regulate ciliogenesis (Hamze-Komaiha et al., 2016). Together, the data thus

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point out that SHIP2 is both a PI(3,4,5)P3 and a PI(4,5)P2 5-phosphatase allowing the two PIs to play specific functions. As many PI 5-phosphatase such as OCRL and SKIP can use these two substrates as well, it is tempting to speculate that the concept could be generalized to other PI 5-phosphatases.

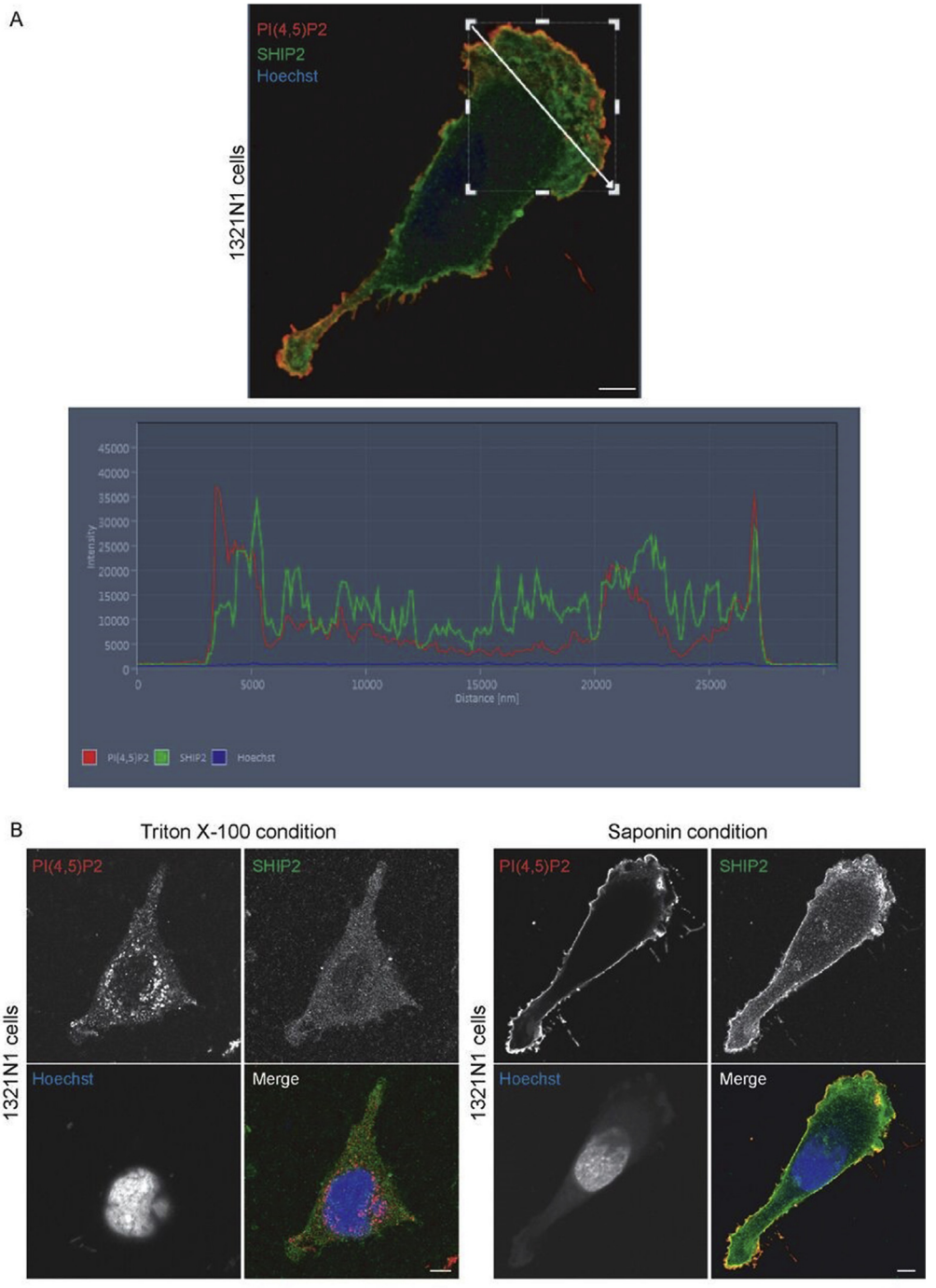

Fig. 1. (A) 1321 N1 cells were plated on coverslips and kept in culture in the presence of 10% serum for 24 h. The cells were fixed and processed as described in (Elong Edimo et al., 2016a) for immunostaining made in the presence of "saponin". PI(4,5)P2 immunostaining in red (Streptavidin NL557 conjugated) and SHIP2 in green (Alexa Fluor 488). Shown in (A), the intensity plots for the red and green fluorochromes measured on the white line. Nucleus was stained with Hoechst 33342 in blue. Images were obtained by confocal microscopy (Elong Edimo et al., 2016a). (B) Comparison of SHIP2 staining between the "Triton X-100" and "saponin" method. Scale bar 5 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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5. Both PI 5- phosphatase catalytic activity and localization influence cellular behavior

Specificity of intracellular response is achieved by two major mechanisms: (1) catalytic specificity/activity (Schmid et al., 2004) and allosteric interactions between phosphatase domains recently reported for SHIP2 (Le Coq et al., 2017) and (2) intracellular localization. OCRL, SKIP and SHIP2 (three PI 5-phosphatases that can take both PI(3,4,5)P3 and PI(4,5)P2 as substrate) have different cellular distribution: OCRL targets the plasma membrane, endosomes, lysosomes, Golgi complex and primary cilium (De Matteis et al., 2017). SKIP has been reported at the endoplasmic reticulum, plasma membrane and ruffles (Gurung et al., 2003; Ijuin and Takenawa, 2012). SHIP2 has a perinuclear localization but also co-localized with focal adhesion markers and ruffles (Elong Edimo et al., 2013; Elong Edimo et al., 2016a; Prasad et al., 2001) and with markers of the invadopodium in human breast cancer cells (Sharma et al., 2013). A phosphorylated form of SHIP2 on S132 is also detected in the nucleus in some glioblastoma cells and non-cancer cells such as human thyroid (Elong Edimo et al., 2011).

PI 5-phosphatase such as OCRL or SHIP2 are concentrated in the cytoplasm or in a perinuclear region and do translocate to the plasma membrane or ruffles in EGF or PDGF stimulated cells for SHIP2 (Pesesse et al., 2001; Taylor et al., 2000). SKIP is at the endoplasmic reticulum (ER) in resting conditions and could translocate to membrane ruffles in response to insulin (Gurung et al., 2003; Ijuin and Takenawa, 2003). In our studies, SHIP2 localization in glioblastoma 1321 N1 cells was very much influenced by the staining conditions (Elong Edimo et al., 2016a): The use of the Hammond staining conditions to preserve plasma membrane integrity (Hammond et al., 2009) allows the localization of PI(4,5)P2 at the plasma membrane as well as SHIP2. Co-staining of SHIP2 and PI(4,5)P2 antibodies is detected with some co-localization depending on the cell area (Fig. 1A). This could be generalized to other glioblastoma cell lines and primary cells (Elong Edimo et al., 2016a). A typical staining of SHIP2 and PI(4,5)P2 in primary human glioblastoma is shown in Fig. 2. When the staining is performed in the presence of Triton X-100 to permeabilize cells as reported before (Elong Edimo et al., 2011; Elong Edimo et al., 2013), the detection of SHIP2 in most cells is rarely at the cell periphery but rather perinuclear (for example in 1321 N1 cells in Fig. 1B). SKIP has been reported to be associated to the ER using the “Triton X-100” staining protocol (Gurung et al., 2003). We confirmed this result in U-87MG cells (Fig. 3). This is in contrast to SHIP2 that shows a cytoplasmic localization in those cells. Both SKIP and SHIP2 immunoreactivity is detected at the plasma membrane ruffles using the “saponin” staining method (Fig. 3). The minimal interpretation of these results is that a fraction of SKIP and SHIP2 in U87MG is present at the plasma membrane of cells kept in 10% serum. In agreement with the immunofluorescence data, by measuring PIs (after ^{32}P labelling), SHIP2 was shown to be active in 1321 N1 cells already in starved cells (Elong Edimo et al., 2011; Elong Edimo et al., 2013). It is possible that the ratio of plasma membrane ruffles to cytosolic SHIP2 would be influenced by agonist stimulation (EGF or serum), phosphorylation events or protein:protein interaction. The same conclusion could apply for SKIP by comparing SKIP at the plasma membrane and at the ER. Interestingly, the data also show that the two PI 5-phosphatases could be present in ruffles possibly acting on the same substrate(s).

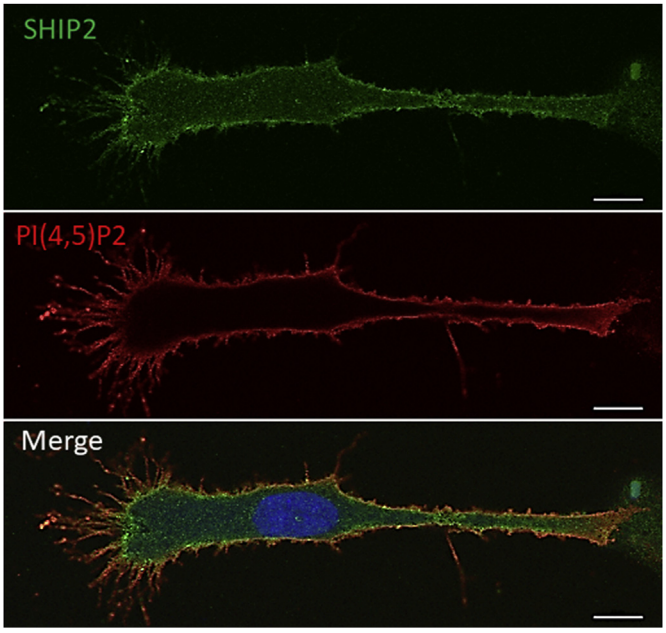

Fig. 2. Immunostaining for PI(4,5)P2 in red (Streptavidin NL557 conjugated) and SHIP2 in green (Alexa Fluor 488) in a primary culture of human glioblastoma. Nucleus was stained was stained with Hoechst 33342 in blue. Data obtained on a Zeiss LSM780 confocal system fitted on an Observer Z1 inverted microscope equipped with alpha Plan-Apochromat 63x/1.46 N.A. oil immersion objective. Methods detailed in (Elong Edimo et al., 2016a). Scale bar 10 μm.

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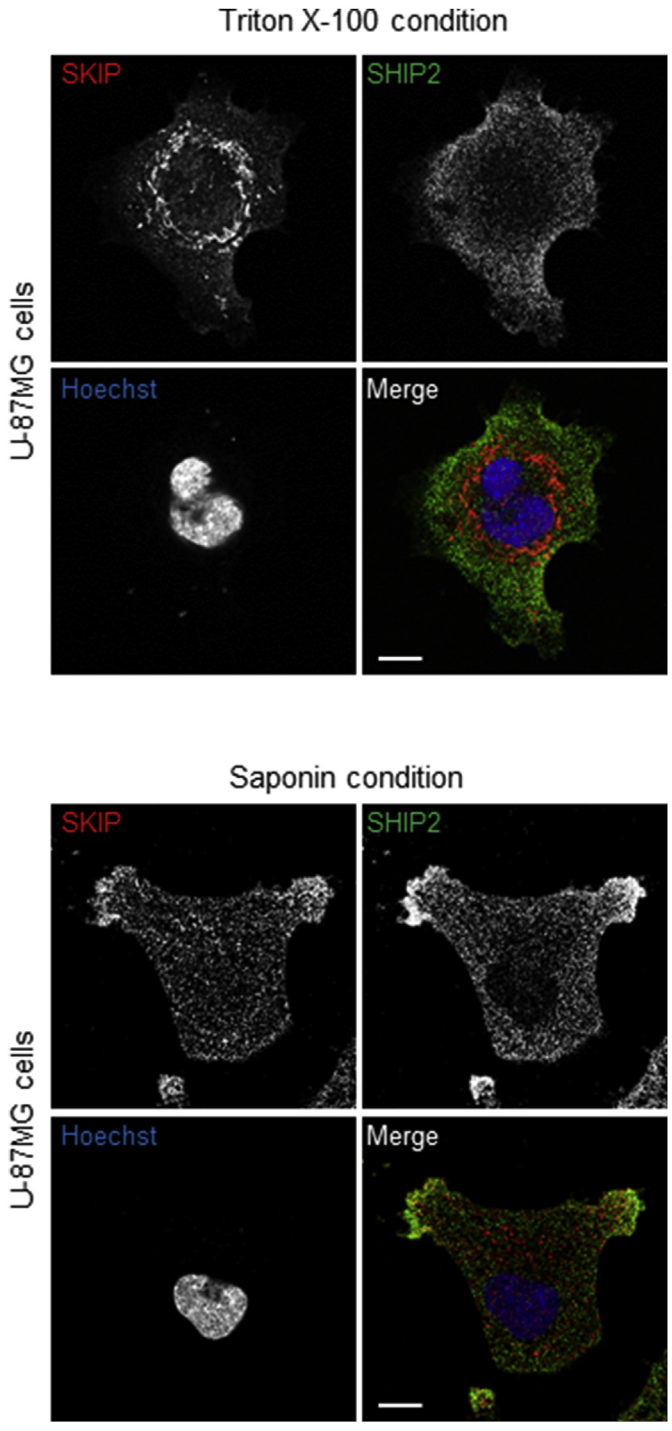

Fig. 3. Comparison of SKIP endogenous immunostaining using "Triton X-100" protocol and the "saponin" protocol.
U-87MG cells were plated on coverslips and kept in culture in the presence of 10% serum for 24 h. The cells were fixed and stained with anti-SKIP (LifeSpan BioSciences, Inc; catalog number LS-B10239) in red (Alexa Fluor 594) and anti-SHIP2 (Novus; catalog number H00003636-M01) in green (Alexa Fluor 488). Comparison of staining between the "Triton X-100" and "saponin" method. (Elong Edimo et al., 2016a). Nucleus was stained with Hoechst 33342 in blue. Images were obtained by confocal microscopy using Zeiss LSM780 at 63x/NA 1.46. Scale bar 10 μm.

6. Conclusions

Cytoskeletal remodeling plays a fundamental role in cell motility, polarity, invasion and even metabolic reprogramming downstream of growth factor receptors. PI 5-phosphatase may control the abundance of PIs substrates (PI(3,4,5)P3 and PI(4,5)

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P2) or products (PI(3,4)P2 and PI4P). PI(3,4)P2 has been recently connected to key proteins involved in cell migration and invasion (Mena, Tks5 or lamellipodin). Both SHIP1 and SHIP2 catalyze the production of PI(3,4)P2 with established second messenger functions in cancer cells. PI(4,5)P2, often presented as multifunctional, also controls many steps of cytoskeletal organization via its interaction with a large number of cytoskeletal proteins to regulate migration and invasion of tumor cells. Importantly, PI 5-phosphatases can often take both PI(3,4,5)P3 and PI(4,5)P2 as substrate thereby connecting cytoskeletal remodeling to different mechanisms and sometimes to different final responses. Depending on the cell type, PI 5-phosphatase in particular SHIP2 in glioblastoma may have opposite effects on cell motility. This concept is somehow comparable to the role of the protein tyrosine phosphatase PTP1B in signaling: PTP1B was shown to promote cell adhesion and motility in fibroblasts and several tumor cell lines. In contrast an inhibitory role has been described in glioblastoma multiforme tumor cell invasion in mice (Arregui et al., 2013).

Acknowledgements

This work was supported by grants from the « Fonds de la Recherche Scientifique Médicale » (FRSM) (PDRT.004.13), “Université Libre de Bruxelles” and Télévie (Belgium) (n° 7.4534.15). ARR is supported by a Télévie grant (Belgium).

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