



Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

Journal homepage: www.elsevier.com/locate/apjr

Document heading 10.1016/S2305-0500(13)60086-X

Role of the phosphoinositide signal transduction pathway in the endometrium

Vincenza Rita Lo Vasco*

Department Sensitive Organs, Policlinic Umberto I, viale del Policlinico 155 – 00161 Rome Faculty of Medicine and Odontoiatry, Sapienza University of Rome, Italy

ARTICLE INFO

Article history:

Received 18 July 2012

Received in revised form 20 July 2012

Accepted 25 August 2012

Available online 20 September 2012

Keywords:

Abortion

Women's right

United States

ABSTRACT

The regulation of calcium concentration triggers physiological events in all cell types. Unregulated elevation in calcium concentrations is often cytotoxic. In fact, uncontrolled calcium levels alter proteins' function, apoptosis regulation, as well as proliferation, secretion and contraction. Calcium levels are tightly regulated. A great interest was paid to signal transduction pathways for their role in mammalian reproduction. The role of phosphoinositide (PI) signal transduction pathway and related phosphoinositide-specific phospholipase C (PI-PLC) enzymes in the regulation of calcium levels was actively studied and characterized. However, the role of PI signaling and PI-PLC enzymes in the endometrium is far to be completely highlighted. In the present review the role of PI, the expression of selected PI-PLC enzymes and the crosstalk with further signaling systems in the endometrium will be discussed.

1. Introduction

The regulation of calcium concentration triggers physiological events in all cell types[1]. Unregulated elevation in calcium concentrations is often cytotoxic[2]. In fact, uncontrolled calcium levels alter proteins' function, apoptosis regulation, as well as proliferation, secretion and contraction. Calcium levels are tightly regulated by signal transduction pathways, including the phosphoinositide (PI) system [1,3].

A great interest was paid to PIs for their role in mammalian reproduction. In myometrium, the role of PI signal transduction pathway and related Phosphoinositide-specific Phospholipase C (PI-PLC) enzymes in the regulation of calcium levels was actively studied and characterized. Downstream products of PI-PLC activity are known to play a role in the contraction of uterine smooth muscle cells following the activation of muscarinic and oxytocin receptors in the guinea pig myometrium[4,5]. PI-PLC ζ enzyme is exclusively present in the spermatid cells. PI-PLC δ 4 is required for the zona pellucida-induced acrosome reaction[6]. In mice, the mutation of the gene which codifies for PI-plc β 1 reduces the acrosome reaction

rate, the fertilization rate and the embryo developmental rate[7].

Less is known about the role of PI signaling and PI-PLC enzymes in the endometrium. Reports from the '80s indicated that the PI signal transduction pathway plays a role also in the endocrine regulation of endometrium. In our recent report, we suggested that PI-PLC enzymes might be involved in endometriosis. In the present review the role of PI, the expression of selected PI-PLC enzymes and the crosstalk with further signaling systems in the endometrium will be discussed.

2. PI signal transduction pathway

PIs are minority acidic phospholipids present in the membranes of eukaryotic cells[8]. The principal role of PIs is instructional. Each cell membrane compartment uses a characteristic PI's pattern, which attracts a specific panel of proteins. PIs regulate the activities of integral membrane proteins. Many ion channels of the plasma membrane are regulated by the levels of phosphatidylinositol 4,5-bisphosphate (PIP₂), which is the principal substrate of receptor-stimulated PI-PLC[9]. Over 150 different molecules can activate PI-PLC. Once activated, the PI-PLC cleaves the polar head group of PIP₂, a structural component of the plasma membrane[9–11], generating inositol trisphosphate (IP₃) and diacylglycerol (DAG), both crucial molecules in

*Corresponding author: Department Sensitive Organs, Policlinic Umberto I, viale del Policlinico 155–00161 Rome, Faculty of Medicine and Odontoiatry, Sapienza University of Rome.

E-mail: ritalovasco@hotmail.it

signal transduction^[12]. IP₃, a small water-soluble molecule, diffuses rapidly to the cytoplasm, where it releases calcium from the endoplasmic reticulum. DAG remains bound to the membrane, exerting two potential signalling roles^[13]. First, it can be further cleaved to release arachidonic acid, which either acts as a messenger or is used in the synthesis of eicosanoids. Therefore, DAG is probably involved in the inflammation cascade. Moreover, recent evidences suggested that PI-PLC might be involved in the inflammation cascade^[14]. Second, DAG activates protein kinase C (PKC). The calcium levels enhancement induced by IP₃ moves PKC to translocate from the cytoplasm to the cytoplasmic face of the plasma membrane^[15]. The translocation activates PKC, which phosphorylate specific serine or threonine residues on further target proteins^[16].

3. PI-PLC family

In eukaryotes, the PI-PLC family comprises a related group of multi-domain proteins^[17]. Thirteen mammalian enzymes were identified, divided into six sub-families on the basis of structure and activation mechanisms: β (1–4), γ (1, 2), δ (1, 3, 4), ϵ (1), ζ (1), and η (2)^[17].

PI-PLC subfamilies share a conserved core architecture containing an *N*-terminal pleckstrin (PH), followed by a series of elongation factor hand motifs (EF), a complex catalytic domain (X–Y) and a *C*-terminal PKC conserved region 2 domain (C2)^[18–20]. In the catalytic domain, the *N*-terminal X region, of 65 aminoacids, binds calcium. The *C*-terminal Y region, of 115 aminoacids, binds primarily the substrate.

Beside this common core, further domains, peculiar to each sub-family, contribute to specific regulatory mechanisms. Isoforms within sub-families share sequence similarity, specific domains, molecular effectors and targets, confirming the versatility of the interactions at the cellular membrane and its proximity.

4. PI-PLC regulation

The PI-PLC family of enzymes covers a broad spectrum of interactions that contribute to membrane recruitment, including binding to PI, interaction with small GTPases from Ras and Rho families or heterotrimeric GTP-binding protein (G protein) subunits, recognition of specific sites in receptor and non-receptor tyrosine kinases and interaction with lipid components of the cell membranes. Two major mechanisms were described in receptor-promoted regulation of PI-PLC enzymes^[18–20].

G proteins: activating molecules, such as hormones and neurotransmitters stimulate G-protein-coupled receptors (GPCRs) to activate ubiquitously expressed members of G protein family. The G protein subunits are classified into subfamilies that couple to different effectors^[21]. Three subunits (α , β , γ) comprise the G proteins. In the inactive state, the G α subunit binds GDP. Receptor activation by ligand stimulates GDP-GTP exchange on the G α subunit, and the activated GTP-bound G α subunit dissociates from the G β γ complex. Both the G α and G β γ subunits can influence the activity of effector molecules. In instance, PI-PLC β isozymes are directly activated by G α -subunits

of the Gq subfamily. G proteins mediate the intracellular signalling by coupling to GPCRs^[22,23]. The tyrosine kinase-activated mitogen-activated protein kinase (MAPK) pathway can be activated by G β γ acting at the Shc protein, by G α q-stimulated PKC acting at Raf, and by a PKC-independent pathway^[24]. GTPase activity in the G β γ subunit promotes re-association of GDP-bound GTPase activity in the subunit with G β γ . The specificity of response to a given ligand in each cell type depends on the expression and coupling of specific receptors, G proteins, and effectors. In addition to direct stimulation by G proteins of effectors that initiate signalling pathways, there can be cross-talk among downstream effectors of these pathways^[25]. Protein kinase A (PKA), activated by G α s stimulation of adenylyl cyclase, can inhibit both PI-PLC activation and the MAPK pathway^[26,27]. Also ion channels can be regulated by phosphorylation catalysed by PKA, PKC and other effectors of the G protein signalling pathway. GTPases: the Ras super-family of small GTPases comprises more than 150 members^[28]. These molecular switches fulfill critical roles in physiologic and pathologic events, including regulation of cell proliferation, differentiation, survival, polarity, shape, movement, regulation of gene expression, vesicle transport, and many other activities. An enormous number of upstream activators and regulators of Ras GTPases were indentified. Upon the other side, many different proteins are downstream effectors of activated GTP-bound forms of Ras GTPases. An extensive crosstalk among pathways involving Ras GTPases results in a complex signalling network. Many GPCR-initiated events also activate Ras GTPases further increasing crosstalk.

Activation of GPCRs that couple to other G proteins (*eg.*, Gi) can also promote the PI signalling. PI-PLC β 2 and PI-PLC β 3 seem to be directly activated by G β γ dimers released from G proteins. The C terminal domain of PI-PLC β subfamily is necessary for membrane association, G α q-dependent activation, and promotion of the GTPase activity of G α q and related α -subunits. G β γ was supposed to interact with both the PH domain and the catalytic core of PI-PLC β 2 and PI-PLC β 3. Growth factors promote PI signalling involving receptor autophosphorylation and generation of phosphotyrosine binding sites for PI-PLC γ enzymes on the activated receptor^[29,30]. Also small GTPases of the Ras superfamily^[31] may activate selected PI-PLC isoforms such as PI-PLC β 2, PI-PLC γ 2, and PI-PLC ϵ ^[32]. PI-PLC β 3 also binds Rac, although with lower affinity.

5. Estrogens and PI-PLC

In rat uterus, estrogen receptors probably mediate rapid signaling events^[33,34], resulting in modification of transcription^[35,36]. Thus, also in this case, the effect of 17 β -estradiol depends on the receptors expressed in each tissue. In immature mice, the estrogen receptors erythrocyte sedimentation rate 1 (ESR1) and ESR2, present in uterine stroma and epithelium, differently respond to 17 β -estradiol, which increases the expression of ESR1 and decreases the expression of ESR2. 17 β -estradiol controls the uterine activities, involving genomic responses, activated by the binding of the estrogen receptors on estrogen-

responsive elements (EREs) present in the promoter region of 17 β -estradiol-regulated genes^[37]. This event is biphasic. Within the first hours, hyperemia and uterine imbibition was observed. After 24–72 h, epithelial cells proliferation and differentiation occurs. Furthermore, 17 β -estradiol-mediated regulation of IGF1 transcription and uterine growth involves the direct binding of ESR1 to EREs. 17 β -estradiol responses in the uterus may also occur independently of ERE-mediated transcription, probably involving non classical ESR1 signaling mechanisms, such as protein-to-protein interactions with transcription factors. A non-genomic signaling from membrane ESR1 contributing to 17 β -estradiol-mediated modulation of gene transcription was also described. In mice uteri, GPER1 mediates the rapid signaling cascade. The activation of GPER in epithelial cells enhances negative regulatory actions in the stroma, inhibiting ERK/12 and ESR1 phosphorylation signals, which are essential for the onset of 17 β -estradiol-regulated uterine epithelial cell proliferation^[37]. ESR1, ESR2 and GPER mediate rapid signaling events activating different downstream pathways, including the MAPK, phosphatidylinositol 3-kinase and PI-PLC pathways, which in turn modulate transcription. 17 β -estradiol promotes rapid effects on the rat endometrium through activation of PI-PLC mediated PI hydrolysis and requires nucleocytoplasmic shuttle of ESR1. GPER probably plays an additive role to this event. Membrane initiated steroid signaling was suggested to be involved in benign and malignant cells of several tissues. These effects occur from seconds to minutes after administration of 17 β -estradiol and involve the rapid activation of many signaling molecules (16, 19, 41), including PI-PLC enzymes. Treatment with 17 β -estradiol induced a rapid increase on total (3H)-inositol phosphate accumulation in the endometrium mediated by PI-PLC activation. Pharmacological inhibition of PI-PLC with the specific inhibitor U73122 decreased the total (3H)-inositol phosphate accumulation. Pre-treatment with the estrogen receptors antagonist ICI 182, 780 blocked the total (3H)-inositol phosphate accumulation induced by 17 β -estradiol. These data indicate that ESR1 acts as an upstream regulator for total (3H)-inositol phosphate accumulation. GPER also seems to contribute to PI-PLC pathway activation in uterus, and mRNA for GPER was detected in the endometrium from rat in estrus. *In vivo* administration of G-1, a selective agonist of GPER, failed to activate expression of Wnt-4, Frizzled-2, IGF-1 or cyclin E1 in uterus^[38]. GPER may play a role in uterine physiology, since G-1 and ICI 182, 780^[39,40] induced a rapid time-dependent increase in total (3H)-inositol phosphate accumulation in the endometrium. The G-1-mediated effects were blocked by pretreatment of the endometrium with G15 and PI-PLC inhibitors, suggesting that the axis GPER1-PLC is also involved in this intracellular pathway.

6. PI-PLC isoforms in the human endometrium

Many studies were performed to investigate each PI-PLC isoform and the interacting molecules, but their potentialities are far to be highlighted. In our previous studies, we identified a peculiar panel of expressed PI-PLC isoforms in normal human endometrium, namely PI-PLC

β 1, β 3, δ 1 and δ 3 isoforms. However, the expression differed in endometriosis tissues with respect to normal endometrium. In women affected with endometriosis, PI-PLC β 1, PI-PLC β 3 and PI-PLC δ 3 were expressed in the eutopic endometrium. PI-PLC β 1, and PI-PLC β 3 were expressed in ectopic tissues. PI-PLC δ 1 was expressed exclusively in the uterosacral ligament localization of the ectopic tissue, indicating that the expression of PI-PLC enzymes differs in the normal endometrium of unaffected woman compared to endometriosis affected patients. The expression levels of PI-PLC β 1 were higher in the normal endometrium. The concentration of PI-PLC β 1 was moderately lower in the eutopic endometrium and much lower in the ectopic tissue. Remarkably, different expression of PI-PLC β 1 was also described during astrocyte activation after lipopolysaccharide (LPS) stimulation^[41,42], suggesting its involvement in the cascade of events following inflammatory stimulus. In endometriosis, the PI-PLC β 1 transcript decreases in the ectopic endometrium, probably related to dysregulation of the activity that PI-PLC β 1 plays in the cell cycle control. However, the role of PI-PLC β 1 remains to be elucidated. The levels of PI-PLC β 3 resulted higher in the endometriotic tissue localized in the uterosacral ligament compared to the normal, to the eutopic endometrium and to the ectopic localization in the ovarian cyst. PI-PLC β 3 findings were controversial compared to our previous studies. In fact, down-regulation was described during astrocyte activation after LPS stimulation^[8]. PI-PLC δ 1 was down-regulated exclusively in endometriosis. PI-PLC δ 3, expressed in the normal and eutopic endometrium, was not expressed in the ectopic tissues independently from the establishment site. That is an interesting point in that one endometriosis susceptibility locus was localized on chromosome 17 (17q21)^[43], a localization consistent with the region where PLCD3 (OMIM *608795), the gene which codifies for PI-PLC δ 3, maps. The endometriosis susceptibility locus on 17q21 might be related to PLCD3 mapping and PI-PLC δ 3 might be involved in the disease.

7. Molecular function and regulation of PI-PLC enzymes expressed in the human endometrium

Literature data lack about the specific role and regulation of PI-PLC β and γ in the human endometrium, although some isoforms belonging to these sub-families resulted expressed. However, the regulation mechanism of some isoforms were analysed in other tissues. Enzymes in the PI-PLC β sub-family share the structural features present in other sub-families, and are characterized by elongated C-terminus about 450 aminoacidic residues long, containing interacting sites for Gq and for other functions such as membrane binding and nuclear localization^[17,28]. In mammals, splicing variants for each PI-PLC β isoform were identified. These enzymes are regulated by G proteins with high GTPase stimulating (GAP) activity. Mammalian PI-PLC β enzymes are differentially distributed in tissues. PI-PLC β 1 is the most widely expressed, especially in specific regions of the brain. PI-PLC β 1 exists as alternative splicing variants PI-PLC β 1a and β 1b, which differ in C-terminal residues^[39]. In the cytoplasm, PI-PLC β isoforms function as effectors for receptors belonging to the

rhodopsin superfamily. They are activated by a variety of stimuli and require special combinations of $G\alpha$ and $G\beta\gamma$ subunits to couple to the effector. Phosphatidic acid (PA) can activate PI-PLC β enzymes, suggesting a role for PA in the regulation of GPCR signal transduction mediated through PI-PLC β 1 homodimerization[44]. With the exception of PI-PLC β 4, PI-PLC β isozymes are also activated by $G\beta\gamma$ dimers[45–47]. The relative sensitivity of PI-PLC β enzymes to $G\beta\gamma$ subunits differs from that to $Gq\alpha$ subunits. PI-PLC β 1 is the least sensitive to $G\beta\gamma$ [11,45,46]. The $G\beta\gamma$ dimer interacts with PI-PLC β 1, β 3, and with high affinity PI-PLC β 2[48]. $G\alpha_q$ subunit seems to be essential for PI-PLC β activation and cannot be replaced by $G\beta\gamma$. Two different regions of PI-PLC β interact with $Gq\alpha$ and $G\beta\gamma$ subunits. Male and female *Plcb1* knock-out mice exhibited impaired reproductive behaviour. Interestingly, female mice's reduced fertility was probably related to abnormal control of ovulation, whilst the impairment of male mice infertility is probably due to abnormal behaviour. Noteworthy, hormones regulate the reproductive processes through GPCR, which activate a number of enzymes, including PI-PLC β .

PI-PLC δ sub-family is considered the most basic due to its simple structure, and probably was the first to appear in primitive eukaryotes. PI-PLC δ sub-family is the most sensitive to calcium concentrations[49,50]. Tissue transglutaminase II (TGII) associates and activates PI-PLC δ 1 *in vitro*[51]. TGII has both transglutaminase activity and GTPase activity. Expression of TGII mutants lacking the interaction with α -adrenoreceptor (α AR) or PI-PLC δ 1 reduced the increase in intracellular calcium[52], suggesting the involvement of PI-PLC δ 1 in α AR signaling. The Rho GTPase-activating protein (RhoGAP) p122 directly activates PI-PLC δ 1[53]. Microinjection of the GAP domain of p122 suppressed the formation of stress fibers and focal adhesions induced by lysophosphatidic acid, suggesting a specific GTPase-activating function of p122 for Rho[54]. Therefore, p122 synergistically functions as a RhoGAP and an activator of PI-PLC δ 1 *in vivo*, inducing cytoskeletal reorganization.

Plcd1 null mice presented with hair loss associated with abnormal hair follicle structures and epidermal hyperplasia in interfollicle epidermis[55], common findings in skin inflammation. The expression of pro-inflammatory cytokines, such as IL-1 β and IL-6, significantly increases and infiltration of immune cells is observed[56]. Therefore, lack of *Plcd1* results in induction of skin inflammation, successfully treated with anti-inflammatory reagents. The *Plcd1* null mouse was compared to nude mouse[57], in which *Foxn1* gene is spontaneously mutated[58], leading to insufficient hair keratin mHa3 expression and abnormal hair shaft structures[59]. Expression of mHa3 was also decreased in the skin of *Plc* δ 1 null mice. In addition, exogenously expressed *Foxn1* induced *Plcd1* expression. *Plcd1* was abundantly expressed in hair follicles of control mice, whereas faint expression was observed in hair follicles of nude mice. These results confirm that *Foxn1* acts as an upstream regulator of PI-PLC δ 1. On the other hand, PI-PLC δ 1 may be involved in the *Foxn1*/mHa3 axis regulation. Although *Plcd3* null mice showed no abnormalities, *Plcd1/d3* double null mice resulted in embryonic lethality at E11.5–E13.5 due to defects of

placental development[16], including reduction of vessels number in the labyrinth architecture. Interestingly, *Plcd1/d3* double null mice embryos supplied with a normal placenta by the tetraploid aggregation method[60] survived beyond E14.5. Placentas of rescued *Plc* δ 1/– δ 3 double null mice embryos contained many maternal and embryonic vessels, indicating that the embryonic lethality is caused by a defect in trophoblasts. Therefore, PI-PLC δ 1 and PI-PLC δ 3 are essential in trophoblast/placenta development. Interestingly, homozygous deletions of the murine chromosome 9 region containing a few genes, including *Plcd1*, result in phenotype abnormalities, such as alopecia in both genders and male infertility.

PI-PLC δ 1 was also suggested to be an anti-oncogene. *PLCD1*, the human gene which codifies for PI-PLC δ 1, maps in 3p22 chromosomal region, which is frequently deleted in esophageal squamous cell carcinoma (ESCC) [61]. Absence of *PLCD1* was frequently detected in both primary ESCCs and ESCC cell lines. Introduction of *PLCD1* into ESCC cells suppresses the tumorigenic ability. In addition, down-regulation of PI-PLC δ 1 protein seems to significantly correlate with ESCC metastasis. The observation of hyperprasia and enhanced proliferation in the skin of *Plcd1* null mice suggests that PI-PLC δ 1 might play a suppressive role in the development and progression of ESCC. Moreover, *DLC-1*[62], a gene frequently deleted in liver cancer, shares high homology with the rat p122 RhoGAP[62]. *DLC-1* inhibited human cancer cell growth and *in vivo* tumorigenicity in nude mice. Therefore, p122 might function as an anti-oncogene by synergic interaction with PI-PLC δ 1 and by modulating the Rho-mediated reorganization of cytoskeleton[63]. PI-PLC δ 1 has both nuclear export and import sequences that contribute to its shuttling between the cytoplasm and nucleus[63]. PI-PLC δ 1 is generally found in the cytoplasm of quiescent cells, and may also localize in nuclear structures in the G1/S boundary of the cell cycle[64]. Suppression of PI-PLC δ 1 by siRNA increases the level of cyclin E, a key regulator of the G1/S boundary, alters S-phase progression, and inhibits cell proliferation[65]. In addition, transient expression of PI-PLC δ 1 suppressed the expression of cyclin E at the G1/S boundary. Therefore, PI-PLC δ 1 probably plays a role in cell cycle progression.

8. Conclusion

Further studies are required to elucidate the role of PI-PLC isoforms in endometrium, with special regard to the crosstalk among the expressed enzymes. Moreover, the extensive network of interactions with G proteins and oestrogens suggests that the PI-PLC enzymes might act as crucial players during the activities of normal endometrium. However, research efforts will be necessary to highlight the involvement of PI-PLC pathway in endometriosis. In fact, the different expression of PI-PLC enzymes in normal endometrium with respect to endometriotic counterpart and in different localizations of the shed tissue suggests that the PI signal transduction pathway might be related to endometriosis pathogenesis. Our previous studies about the role of PI-PLC in inflammation suggest that the PI transduction system might act a role interfering with the

inflammation cascade widely described in the disease. This interesting hypothesis opens the way to further studies addressed to analyze the activity and regulation of PI–PLC isoforms in the endometrium, and to delineate their relationship with inflammation, representing a promising approach in order to develop novel molecular therapeutic strategies for endometriosis and, probably, for other endometrial diseases. Finally, studies addressed to identify signal transduction pathways interacting upstream and downstream with the PI–PLC system might allow the identification of further molecular targets useful to design novel molecular strategies for therapy.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Berridge M. Inositol triphosphate and calcium signalling. *Nature* 1993; **361**: 315–325.
- [2] Annunziato L, Amoroso S, Pannaccione A, Cataldi M, Pignataro G, D'Alessio A, et al. Apoptosis induced in neuronal cells by oxidative stress: role played by caspases and intracellular calcium ions. *Toxicol Lett* 2003; **139**(2–3): 125–133.
- [3] Berridge MJ. Calcium signalling and cell proliferation. *BioEssays* 1995; **17**: 491–500.
- [4] Marc S, Leiber D, Harbon S. Carbachol and oxytocin stimulate the generation of inositol phosphates in the guinea pig myometrium. *FEBS Lett* 1986; **26**: 201(1): 9–14.
- [5] Bennet PJ, Moatti JP, Mansat A, Ribbes H, Cayrac JC, Pontonnier F, et al. Evidence for the activation of phospholipases during acrosome reaction of human sperm elicited by calcium ionophore A23187. *Biochim Biophys Acta* 1987; **919**(3): 255–265.
- [6] Fukami K, Nakao K, Inoue T, Kataoka Y, Kurokawa M, Fissore RA, et al. Requirement of phospholipase Cdelta4 for the zona pellucida-induced acrosome reaction. *Science* 2001; **292**(5518): 920–923.
- [7] Choi D, Lee E, Hwang S, Jun K, Kim D, Yoon BK, et al. The biological significance of phospholipase C beta 1 gene mutation in mouse sperm in the acrosome reaction, fertilization, and embryo development. *J Assist Reprod Genet* 2001; **18**(5): 305–310.
- [8] Falkenburger BH, Jensen JB, Dickson EJ, Suh BC, Hille B. Phosphoinositides: lipid regulators of membrane proteins. *J Physiol* 2010; **588**(Pt 17): 3179–3185.
- [9] Berridge MJ, Irvine RF. Inositol triphosphate, a novel second messenger in cellular. *Nature* 1984; **312**: 315–321.
- [10] Kirk CJ, Bone EA, Palmer S, Michell RH. The role of phosphatidylinositol 4,5 biphosphate breakdown in cell–surface receptor activation. *J Recept Res* 1984; **4**(1–6): 489–504.
- [11] Smrcka AV, Hepler JR, Brown KO, Sternweis PC. Regulation of polyphosphoinositide-specific phospholipase C activity by purified Gq. *Science* 1991; **251**(4995): 804–807.
- [12] Berridge M J, Irvine R F. Inositol phosphates and cell signaling. *Nature* 1989; **341**: 197–205.
- [13] Prokazova NV, Samoilova NN, Golovanova NK, Gracheva EV, Korotaeva AA, Andreeva ER. Lipid second messengers and cell signaling in vascular wall. *Biochemistry (Mosc)* 2007; **72**(8): 797–808.
- [14] Lo Vasco VR, Fabrizi C, Fumagalli L, Cocco L. Expression of phosphoinositide specific phospholipase C isoenzymes in cultured astrocytes activated after stimulation with Lipopolysaccharide. *J Cell Biochem* 2010; **109**(5): 1006–1012.
- [15] Nishizuka Y. Studies and perspectives of the protein kinase c family for cellular regulation. *Cancer* 1989; **63**(10): 1892–1903.
- [16] Nakamura Y, Hamada Y, Fujiwara T, Enomoto H, Hiroe T, Tanaka S, et al. Phospholipase C–delta1 and –delta3 are essential in the trophoblast for placental development. *Mol Cell Biol* 2005; **25**: 10979–10988.
- [17] Suh PG, Park J, Manzoli L, Cocco L, Peak JC, Katan M, et al. Multiple roles of phosphoinositide–specific phospholipase C isozymes. *BMB Reports* 2008; **41**: 415–434.
- [18] Ellis MV, Katan M. Mutations within a highly conserved sequence present in the X region of phosphoinositide–specific phospholipase C–delta 1. *Biochem J* 1995; **307**: 69–75.
- [19] Williams BL, Irvin BJ, Sutor SL, Chini CC, Yacyszyn E, Bubeck Wardenburg J, et al. Phosphorylation of Tyr319 in ZAP–70 is required for T–cell antigen receptor–dependent phospholipase C–gamma1 and Ras activation. *EMBO J* 1999; **18**(7): 1832–1844.
- [20] Ellis MV, James SR, Perisic O, Downes CP, Williams RL, Katan M. Catalytic domain of phosphoinositide–specific phospholipase C (PLC). Mutational analysis of residues within the active site and hydrophobic ridge of plcdelta1. *J Biol Chem* 1998; **273**: 11650–11659.
- [21] Exton JH. Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. *Annu Rev Pharmacol Toxicol* 1996; **36**: 481–509.
- [22] Conklin BR, Bourne HR. Structural elements of G alpha subunits that interact with G beta gamma, receptors, and effectors. *Cell* 1993; **73**(4): 631–641.
- [23] Hedin KE, Duerson K, Clapham DE. Specificity of receptor–G protein interactions: searching for the structure behind the signal. *Cell Signal* 1993; **5**(5): 505–518.
- [24] van Biesen T, Hawes BE, Raymond JR, Luttrell LM, Koch WJ, Lefkowitz RJ. G(o)–protein alpha–subunits activate mitogen–activated protein kinase via a novel protein kinase C–dependent mechanism. *J Biol Chem* 1996; **271**(3): 1266–1269.
- [25] Lopez–Ilasaca M, Crespo P, Pellici PG, Gutkind JS, Wetzker R. Linkage of G protein–coupled receptors to the MAPK signaling pathway through PI 3–kinase gamma. *Science* 1997; **275**(5298): 394–397.
- [26] Chen J, Iyengar R. Suppression of Ras–induced transformation of NIH 3T3 cells by activated G alpha s. *Science* 1994; **263**(5151): 1278–1281.
- [27] Sanborn BM. Ion channels and the control of myometrial electrical activity. *Semin Perinatol* 1995; **19**(1): 31–40.
- [28] Bunney TD, Katan M. PLC regulation: emerging pictures for molecular mechanisms. *Trends Biochem Sci* 2011; **36**(2): 88–96.
- [29] Wang H, Reiser G. Signal transduction by serine proteinases in astrocytes: regulation of proliferation, morphologic changes, and survival via proteinase–activated receptors. *Drug Devel Res* 2003; **60**: 43–50.
- [30] Paterson A, Boyer JL, Watts VJ, Morris AJ, Price EM, Harden TK. Concentration of enzyme–dependent activation of PLC–beta 1 and PLC–beta 2 by G alpha 11 and beta gamma–subunits. *Cell Signal* 1995; **7**(7): 709–720.
- [31] Jezyk MR, Snyder JT, Gershberg S, Worthylake DK, Harden TK, Sondek J. Crystal structure of Rac1 bound to its effector phospholipase C–beta2. *Nat Struct Mol Biol* 2006; **13**(12): 1135–1140.

- [32] Vicinanza M, D'Angelo G, Di Campli A, De Matteis MA. Function and dysfunction of the PI system in membrane trafficking. *EMBO J* 2008; **27**: 2457–2470.
- [33] Manavathi B, Kumar R. Steering estrogen signals from the plasma membrane to the nucleus: two sides of the coin. *J Cell Physiol* 2006; **207**(3): 594–604.
- [34] Song RX, Santen RJ. Membrane initiated estrogen signaling in breast cancer. *Biol Reprod* 2006; **75**(1): 9–16.
- [35] Björnström L, Sjöberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol Endocrinol* 2005; **19**(4): 833–842.
- [36] Ma L, Pei G. Beta-arrestin signaling and regulation of transcription. *J Cell Sci* 2007; **120**(Pt 2): 213–218.
- [37] Konigame VC, Siu ER, Royer C, Lucas TF, Porto CS, Abdalla FM. Estrogen receptors mediate rapid activation of phospholipase C pathway in the rat endometrium. *Steroids* 2011; **76**(14): 1582–1589. Epub 2011 Oct 8.
- [38] Otto C, Rohde-Schulz B, Schwarz G, Fuchs I, Klewer M, Brittain D, et al. G protein-coupled receptor 30 localizes to the endoplasmic reticulum and is not activated by estradiol. *Endocrinology* 2008; **149**(10): 4846–4856. Epub 2008 Jun 19.
- [39] Bologa CG, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, et al. Virtual and biomolecular screening converge on a selective agonist for GPR30. *Nat Chem Biol* 2006; **2**(4): 207–212.
- [40] Filardo EJ, Quinn JA, Bland KI, Frackelton AR Jr. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol Endocrinol* 2000; **14**(10): 1649–1660.
- [41] Lo Vasco VR, Fabrizi C, Artico M, Cocco L, Billi AM, Fumagalli L, et al. Expression of phosphoinositide-specific phospholipase C isoenzymes in cultured astrocytes. *J Cell Biochem* 2007; **100**(4): 952–959.
- [42] Lo Vasco VR, Leopizzi M, Chiappetta C, Businaro R, Polonia P, Della Rocca C, et al. Expression of Phosphoinositide-specific Phospholipase C enzymes in normal endometrium and in endometriosis. *Fertility Sterility* 2012. epub 17th, May 2012
- [43] Kim H, Suh PG, Ryu SH, Park SH. Assignment of the human PLC- δ -3 gene (PLCD3) to human chromosome band 17q21 by fluorescence *in situ* hybridization. *Cytogenet Cell Genet* 1999; **87**: 209–210.
- [44] Ross EM, Mateu D, Gomes AV, Arana C, Tran T, Litosch I. Structural determinants for phosphatidic acid regulation of phospholipase C- β 1. *J Biol Chem* 2006; **281**: 33087–33094.
- [45] Park D, Jhon DY, Lee CW, Ryu SH, Rhee SG. Removal of the carboxyl-terminal region of phospholipase C- β 1 by calpain abolishes activation by G α q. *J Biol Chem* 1993; **268**: 3710–3714.
- [46] Lee CW, Lee KH, Lee SB, Park D, Rhee SG. Regulation of phospholipase C- β 4 by ribonucleotides and the α subunit of Gq. *J Biol Chem* 1994; **269**: 25335–25338.
- [47] Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P. Isozyme-selective stimulation of phospholipase C- β 2 by G protein β gamma-subunits. *Nature* 1992; **360**: 684–686.
- [48] Runnels LW, Scarlata SF. Determination of the affinities between heterotrimeric G protein subunits and their phospholipase C- β effectors. *Biochemistry* 1999; **38**: 1488–1496.
- [49] Kim YH, Park TJ, Lee YH, Baek KJ, Suh PG, Ryu SH, et al. Phospholipase C- δ 1 is activated by capacitative calcium entry that follows phospholipase C- β activation upon bradykinin stimulation. *J Biol Chem* 1999; **274**: 26127–26134.
- [50] Allen V, Swigart P, Cheung R, Cockcroft S, Katan M. Regulation of inositol lipid-specific phospholipase δ by changes in Ca^{2+} ion concentrations. *Biochem J* 1997; **327**: 545–552.
- [51] Feng JF, Rhee SG, Im MJ. Evidence that phospholipase δ 1 is the effector in the Gh (transglutaminase II)-mediated signaling. *J Biol Chem* 1996; **271**: 16451–16454.
- [52] Kang SK, Kim DK, Damron DS, Baek KJ, Im MJ. Modulation of intracellular Ca^{2+} via α (1B)-adrenoreceptor signaling molecules, G α (h) transglutaminase II) and phospholipase C- δ 1. *Biochem Biophys Res Commun* 2002; **293**: 383–390.
- [53] Homma Y, Emori Y. A dual functional signal mediator showing RhoGAP and phospholipase C- δ stimulating activities. *EMBO J* 1995; **14**: 286–291.
- [54] Sekimata M, Kabuyama Y, Emori Y, Homma Y. Morphological changes and detachment of adherent cells induced by p122, a GTPase-activating protein for Rho. *J Biol Chem* 1999; **274**: 17757–17762.
- [55] Nakamura Y, Fukami K, Yu H, Takenaka K, Kataoka Y, Shirakata Y, et al. Phospholipase C δ 1 is required for skin stem cell lineage commitment. *EMBO J* 2003; **22**: 2981–2991.
- [56] Ichinohe M, Nakamura Y, Sai K, Nakahara M, Yamaguchi H, Fukami K. Lack of phospholipase C- δ 1 induces skin inflammation. *Biochem Biophys Res Commun* 2007; **356**: 912–918.
- [57] Nakamura Y, Ichinohe M, Hirata M, Matsuura H, Fujiwara T, Igarashi T, et al. Phospholipase C- δ 1 is an essential molecule downstream of Foxn1, the gene responsible for the nude mutation, in normal hair development. *FASEB J* 2008; **22**: 841–849.
- [58] Flanagan SP. 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genet Res* 1966; **8**: 295–309.
- [59] Meier N, Dear TN, Boehm T. Wn and mHa3 are components of the genetic hierarchy controlling hair follicle differentiation. *Mech Dev* 1999; **89**: 215–221.
- [60] James RM, Klerkx AH, Keighren M, Flockhart JH, West JD. Restricted distribution of tetraploid cells in mouse tetraploid \rightleftharpoons diploid chimaeras. *Dev Biol* 1995; **167**: 213–226.
- [62] Yuan BZ, Miller MJ, Keck CL, Zimonjic DB, Thorgeirsson S, Popescu NC. Cloning, characterization, and chromosomal localization of a gene frequently deleted in human liver cancer (DLC-1) homologous to rat RhoGAP. *Cancer Res* 1998; **58**: 2196–2199.
- [63] Yamaga M, Fujii M, Kamata H, Hirata H, Yagisawa H. Phospholipase C- δ 1 contains a functional nuclear export signal sequence. *J Biol Chem* 1999; **274**: 28537–28541.
- [64] Stallings JD, Tall EG, Pentylala S, Rebecchi MJ. Nuclear translocation of phospholipase C- δ 1 is linked to the cell cycle and nuclear phosphatidylinositol 4,5-bisphosphate. *J Biol Chem* 2005; **280**: 22060–22069.
- [65] Stallings JD, Zeng YX, Narvaez F, Rebecchi MJ. Phospholipase C- δ 1 is linked to proliferation, DNA synthesis and cyclin E levels. *J Biol Chem* 2008; **283**: 13992–14001.
- [61] Fu L, Qin YR, Xie D, Hu L, Kwong DL, Srivastava G, et al. Characterization of a novel tumor-suppressor gene PLC δ 1 at 3p22 in esophageal squamous cell carcinoma. *Cancer Res* 2007; **67**(22): 10720–10726.