Critical role for classical PKC in activating Akt by phospholipase A2-modified LDL in monocytic cells

Stefan Preiß, Dmitry Namgaladze, Bernhard Brüne *

Faculty of Medicine, Institute of Biochemistry I, Johann Wolfgang Goethe-University, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

Received 10 July 2006; received in revised form 5 December 2006; accepted 22 December 2006

Available online 30 December 2006

Time for primary review 28 days

Abstract

Objective: Modification of low density lipoprotein (LDL) by phospholipases confers pro-atherogenic properties, although signalling pathways of phospholipase-modified LDL (PLA-LDL) remain obscure. We questioned whether members of the protein kinase C (PKC) family are involved in PLA-LDL-induced Akt phosphorylation and survival of THP-1 monocytic cells.

Methods: Akt phosphorylation in THP-1 cells was monitored by Western analysis. To modulate PKC expression cells were transfected with dominant-negative enhanced green fluorescent protein linked PKCα (PKCα-EGFP K368R) and PKCβ (PKCβ-EGFP K371M) constructs or with siRNA specific for PKCα/PKCβ using nucleofection technology. Cell survival was assessed by Annexin V/propidium iodide staining or mitochondrial membrane potential measurement with 3,3′-dihexyloxacarbocyanine iodide (DiOC6) using flow cytometry.

Results: Inhibitors of phospholipase C (PLC) or classical PKCs as well as PKC depletion following phorbol ester treatments, blocked Akt phosphorylation in response to PLA-LDL. In contrast, phosphatidylinositol 3-kinase (PI3K) activation by PLA-LDL was insensitive to PKC inhibition. Using RNA interference to knockdown PKCα and overexpression of dominant-negative PKCα as well as PKCβ drastically lowered Akt phosphorylation after PLA-LDL. Moreover, inhibition of PKC attenuated a PLA-LDL-induced survival response towards oxidative stress in THP-1 cells.

Conclusion: We show that PKCα and PKCβ are critical for PLA-LDL-induced Akt phosphorylation and survival in THP-1 monocytic cells. © 2006 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Atherosclerosis; Lipoprotein; Phospholipases; Protein kinase C

1. Introduction

Progression of atherosclerosis is characterized by trapping LDL in the intima of blood vessels where it undergoes oxidative and lipolytic modifications giving rise to its further retention, aggregation as well as generation of chemotactic signals for monocytes [1]. Monocytes invading into the intima differentiate to macrophages, take up modified LDL and acquire a foam cell phenotype which is critical for the development of atherosclerosis [2].

Phagocytic cell survival/apoptosis in atherosclerotic lesions is an important factor modulating atherosclerosis progression. It is suggested that during early stages of the disease enhanced monocyte/macrophage survival promotes lesion progression whereas in advanced atherosclerosis foam cell death contributes to the development of a necrotic core and plaque instability [3].

We recently noticed that phospholipase A2-modified LDL (PLA-LDL) activates Akt in THP-1 monocytic cells although molecular mechanisms remained undefined [4]. Akt is a major pro-survival signalling kinase. Once activated, Akt phosphorylates several proteins participating in modulation of cell death, such as glycogen synthase kinase 3 (GSK3), Bad or caspase 9 [5]. Akt activation occurs via
binding of its PH-domain to 3-phosphorylated phosphoinositides and subsequent phosphorylation at threonine 308 (T308) (catalyzed by phosphoinositide-dependent protein kinase-1 (PDK-1)) and serine 473 (S473) [6,7]. Mechanistically, phosphorylation at S473 is controversially discussed, because several kinases, including integrin-linked kinase (ILK) [8], DNA-dependent protein kinase (DNA-PK) [9], or rictor/mamalian target of rapamycin (mTOR) complex phosphorylate Akt at S473 [10,11].

The PKC enzyme family affects, among other responses, differentiation, cell metabolism, proliferation and apoptosis [12,13]. PKC isoforms are grouped into 3 categories according to their co-factor requirements. Classical PKCs (α, β and γ) require diacylglycerol (DAG) and Ca2+ to be fully active, novel isoforms (δ, ε, η, θ) require only DAG, whereas atypical PKCs (ζ, υ/λ) are activated independently of either, Ca2+ or DAG. Both, classical and novel PKC isoforms are activated by TPA [14]. The involvement of PKC in vascular disease is a subject of intensive investigation [15]. Previous studies revealed that oxidized LDL increases intracellular Ca2+ with subsequent activation of PKC in macrophages, which correlated with a mitogenic effect of oxidized LDL [16,17]. However, the particular PKC isoform(s) being involved and the molecular mechanism of PKC action are still unclear.

In this study we investigated the role of PKC in PLA-LDL-induced activation of Akt in THP-1 monocytes. We show that PLA-LDL-evoked phosphorylation of Akt and Akt-mediated pro-survival pathways in THP-1 cells demand signalling by the classical PKC isoenzymes PKCα and PKCβ.

2. Materials and methods

2.1. Reagents

Snake venom phospholipase A2 (Naja mossambica mossambica, P7778) was from Sigma (Taufkirchen, Germany). Antibodies against phospho-Akt Ser473 (#9271) and total Akt (#9272) were from Cell Signalling Technology (Beverly, MA, USA). PKCα and -β antibodies and the PKCα-EGFP template plasmid were from BD (Heidelberg, Germany). Gö6976, GF109203X, Rottlerin and U73122 were from Alexis Biochemicals (Lausen, Switzerland), while BAPTA-AM was from Calbiochem (San Diego, CA, USA). PKCα-specific siRNA (5′-GCG-UUG-GGU-UGC-UCA-A-3′) was from Ambion (Austin, TX, USA). PKCβ-specific siRNAs were from Ambion (Austin, TX, USA), Qiagen (Hilden, Germany) and Dharmacon (Lafayette, USA). Control siRNA was from Dharmacon. A plasmid containing the PH domain of Akt linked to EGFP (Akt-PH-EGFP) was a gift of Dr. Peter Downes (University of Dundee, Scotland). A plasmid containing PKCβ K371M mutant linked to EGFP (PKCβ-EGFP K371M) was a gift of Dr. Dominique Joubert (Institut de Genomique Fonctionnelle, Montpellier, France).

2.2. Cell culture

THP-1 human acute monocytic leukemia cells were cultured in RPMI 1640 medium supplemented with 10% heat inactivated foetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin and incubated overnight in serum-free medium before treatments. The investigation conformed with the principles outlined in the Declaration of Helsinki.

2.3. DNA mutagenesis

To construct a PKCα kinase-dead K368R mutant we used the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) and the wild-type PKCα-EGFP expression vector as a template.

2.4. Transfection of DNA constructs and siRNA

For transient expression of PKCα-EGFP K368R, PKCβ-EGFP K371M or Akt-PH-EGFP in THP-1 nucleofection technology (Nucleofector II, Amaxa, Cologne, Germany) was used according to instructions provided by the manufacturer. Briefly, 1 × 10⁶ cells were nucleofected with 1 μg plasmid DNA, incubated in complete medium for 32 h (PKCα-EGFP K368R, PKCβ-EGFP K371M) or 8 h (Akt-PH-EGFP) followed by 16 h incubation in serum-free medium. The transfer of PKCα or PKCβ siRNA into THP-1 cells was achieved by nucleofection as well. 1 × 10⁶ cells were nucleofected with 4 μg of PKCα or PKCβ siRNA and incubated in complete medium for 32 h followed by 16 h in serum-free medium.

2.5. LDL isolation and treatment

Human LDL (d=1.02–1.06 g/ml) was isolated from plasma of healthy volunteers by sequential ultracentrifugation and maintained in the presence of 0.2 mM EDTA to prevent oxidation. Endotoxin content of the preparations was less than 0.05 ng/mg LDL (Cambrex Biosciences, Verviers, Belgium). Phospholipolysis was carried out by incubating LDL (2 mg/ml) with 0.5 U/ml Naja mossambica phospholipase A2 and 2 mM CaCl₂ for 2 h at 37 °C. The reaction was stopped by adding 10 mM EGTA.

2.6. Western blot analysis

Following individual treatments, cells were pelleted and lysed in 120 μl of lysis buffer (50 mM Tris, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 10 mM NaF, 1 mM Na₃VO₄, 1 mM PMSF, and protease inhibitor mixture (pH 7.5)), and 50 μg of protein were loaded onto 10% SDS-polyacrylamide gels. Proteins were transferred onto nitrocellulose membranes, followed by blocking and incubations with primary antibodies according to manufacturer instructions. Visualization of immune complexes was by
horseradish peroxidase-labeled secondary antibodies and ECL detection.

2.7. Fluorescence microscopy

To follow intracellular location of the Akt-PH-EGFP construct upon treatment with PLA-LDL, transfected cells (see above) were fixed in 4% para-formaldehyde for 5 min, mounted onto glass slides and analysed using an Axiowert 200 M fluorescent microscope (Zeiss, Frankfurt, Germany).

2.8. Cell death detection

Cells were processed for cell death detection by flow cytometry (FACS Canto, BD Biosciences, Heidelberg, Germany) using the Annexin V-FITC apoptosis detection kit (Beckman Coulter, Krefeld, Germany) according to manufacturer instructions. Cell death was determined as the sum of apoptotic and necrotic cells. Additionally, mitochondrial membrane potential was measured by flow cytometry using the mitochondrial fluorescent dye DiOC₆ (40 nM, 30 min) and cell death was determined as the percentage of cells showing reduced mitochondrial membrane potential.

2.9. Statistical analysis

Data are expressed as mean±SEM. Two treatment groups were compared by the independent Student \( t \) test. Results were considered statistically significant with a value of \( P<0.05 \).

3. Results

3.1. PLC and PKC signalling provokes phosphorylation of Akt in response to PLA-LDL

Previously we have shown phosphorylation of Akt at serine 473 in THP-1 cells following treatments with PLA-LDL [4]. Searching for underlying signalling circuits pointed towards the involvement of phospholipase C (PLC) and protein kinase C (PKC). Incubating THP-1 cells with PLA-LDL resulted in robust phosphorylation of Akt (Fig. 1A). Using U73122, an inhibitor of PLC, or BAPTA-AM in order to chelate intracellular calcium blocked the phospho-Akt signal without affecting expression of Akt itself. In contrast, EGTA, a chelator of extracellular calcium, slightly reduced Akt phosphorylation. Thus, the release of calcium from intracellular stores rather than a calcium influx through plasma membrane channels is necessary for Akt phosphorylation in response to PLA-LDL.

These results imply the formation of second messengers, capable of activating classical PKC isoforms such as calcium and diacylglycerol, as a pre-requisite for Akt activation by PLA-LDL. To further define the role of PKC in PLA-LDL-induced Akt phosphorylation, we used PKC inhibitors (Fig. 1B). PLA-LDL-evoked phosphorylation of Akt was blocked by pre-treatment with Gö6976, an inhibitor of classical PKCs and GF109203X, an inhibitor of classical and novel type PKCs. In contrast, the PKC-delta-specific inhibitor Rottlerin still allowed Akt phosphorylation upon incubation Fig. 1. Role of PLC and PKC in PLA-LDL-induced Akt S473 phosphorylation. (A) THP-1 cells were pre-incubated with 10 μM BAPTA-AM, 1 mM EGTA or 1 μM U73122 for 30 min followed by stimulation with 10 μg/ml PLA-LDL for 10 min. Akt S473 phosphorylation and Akt expression were determined by Western blotting. (B) THP-1 cells were pre-incubated with 0.1 μM Gö6976, 5 μM GF109203X or 5 μM Rottlerin for 30 min followed by stimulation with 10 μg/ml PLA-LDL for 10 min. Levels of phospho-Akt and Akt were determined by Western analysis. Numbers below graphics (A and B) indicate density of pAkt bands normalized to Akt (fold of control, representing the mean of 3 experiments)

(C) THP-1 cells were transfected with Akt-PH-EGFP, and its translocation to the plasma membrane was followed by fluorescence microscopy: (a) unstimulated cells, (b) cells stimulated with 10 μg/ml PLA-LDL for 10 min, (c) cells pretreated with 0.1 μM Gö6976 for 30 min, (d) cells pretreated with 0.1 μM Gö6976 followed by stimulation with 10 μg/ml PLA-LDL for 10 min. Data from Western analysis and microscopy are representative of at least three independent experiments.
with PLA-LDL and none of the PKC inhibitors affected expression of Akt.

Previously we demonstrated that PLA-LDL-evoked Akt phosphorylation is abolished by LY294002 and Wortmannin, and thus is PI3K dependent [4]. To address the question whether classical PKCs might modulate PI3K activation upon PLA-LDL addition, we expressed a plasmid coding the Akt-PH domain fused to EGFP in THP-1 cells. Translocation of this construct to the plasma membrane should reflect PI3K activity [18]. Using fluorescence microscopy we show that PLA-LDL induces membrane translocation of the Akt-PH-EGFP construct (Fig. 1C, part b). Translocation initiated by PLA-LDL was neither affected by Gö6976 (Fig. 1C, part d), nor did Gö6976 alone influence localization of Akt-PH-EGFP (Fig. 1C, part c). This result suggests that inhibition of PLA-LDL-evoked Akt phosphorylation by Gö6976 occurs downstream of PI3K activation.

To further strengthen a role of classical or novel type PKCs in Akt activation, we down-regulated PKC with the phorbol ester TPA. THP-1 cells were incubated with 100 nM TPA for 24 h and further cultured without TPA for 24 or 48 h in order to recover PKC expression (Fig. 2). In controls as well as PKC depleted vs. recovered cells we determined the expression of phospho-Akt, Akt, PKCα and PKCβ upon stimulation with 10 μg/ml PLA-LDL for 10 min.

As expected, TPA depleted PKCα and PKCβ within 24 h. Upon TPA removal, the expression of PKCα and PKCβ returned at 48 h but not at 24 h. Accordingly, phosphorylation of Akt was noticed in controls, was absent in cells with classical PKCs being depleted and reappeared when expression of PKCα and PKCβ had returned at 48 h of the recovery phase, i.e. after removing TPA from the culture medium. So far, pharmacological approaches to block PLC and/or PKC activity or interventions to deplete and recover PKC expression suggest that classical forms of PKC transmit phosphorylation of Akt in response to PLA-LDL.

Fig. 3. Silencing of PKCα attenuates PLA-LDL-induced Akt phosphorylation. (A) THP-1 cells were nucleofected with PKCα-specific siRNA or control siRNA as described under Materials and methods prior to stimulation with 10 μg/ml PLA-LDL for 10 min. Levels of phospho-Akt, Akt, PKCα and PKCβ were monitored by Western blotting. Densitometric analysis of blots shows (B) the ratio of phospho-Akt vs. Akt or (C) the ratio of PKCα vs. Akt. Data are presented as the mean±SEM, n=3. *, P<0.05 vs. PLA-LDL; **, P<0.05 vs. control siRNA transfected cells.
3.2. PKCα and PKCβ are involved in Akt phosphorylation by PLA-LDL

To elucidate which PKC isoform mediates PLA-LDL effects on Akt we used molecular approaches to modulate expression of PKCα and PKCβ. First, we used RNA interference to knockdown PKCα expression. THP-1 cells were nucleofected with PKCα siRNA, stimulated with PLA-LDL and subsequently analyzed for the expression of phospho-Akt, Akt, PKCα and PKCβ (Fig. 3A). To exclude an unspecific effect of the transfection procedure and siRNA itself we applied control siRNA. As shown in Fig. 3A control siRNA neither affected PKC expression nor PLA-LDL-induced Akt phosphorylation. PKCα RNA interference significantly lowered the protein amount of PKCα without having a major effect on PKCβ or Akt expression. Upon cell stimulation with PLA-LDL the signal for phospho-Akt increased in control cells but was largely attenuated in PKCα siRNA-depleted cells. Densitometric analysis of these results is presented in Fig. 3B showing the ratio of phospho-Akt vs. Akt and in Fig. 3C giving the ratio of PKCα vs. Akt, thus underscoring statistical significance of the effects. In a second approach we transiently overexpressed a kinase dead and thus dominant negative mutant of PKCα (PKCα-EGFP K368R) [19] to follow Akt phosphorylation in response to PLA-LDL (Fig. 4A). In THP-1 cells expressing the dominant negative form of PKCα, PLA-LDL-evoked Akt phosphorylation was largely impaired compared to controls. Expression of Akt was slightly altered in PKCα-EGFP K368R expressing cells. As shown in Fig. 4B, densitometric analysis of these results proved a statistical significance.

To examine participation of PKCβ in PLA-LDL-induced Akt phosphorylation we first applied siRNA targeting PKCβ. Unexpectedly, PKCβ siRNA also knockdown PKCα protein. This effect was observed with four independent siRNA constructs targeting different portions of the PKCβ gene (data not shown). To overcome this drawback and to elucidate the role of PKCβ we transiently overexpressed a kinase dead mutant of PKCβ (PKCβ-EGFP K371M) [20]. In THP-1 cells overexpressing this dominant negative form of PKCβ, PLA-LDL-induced Akt phosphorylation was completely abolished (Fig. 4C). Densitometric analysis of these results is presented in Fig. 4D, proving statistical significance.

Corroborating earlier studies, we noticed that 10 μg/ml PLA-LDL supplied together with 1 mM H2O2 for 8 h attenuated cell death promoted by hydrogen peroxide. We noticed that 1 mM H2O2 produced roughly 25% Annexin V

Fig. 4. Expression of PKCα or PKCβ kinase dead mutants reduces Akt phosphorylation. (A) THP-1 cells were transiently transfected with the PKCα-EGFP kinase dead mutant K368R as described under Materials and Methods. Cells expressing PKCα-EGFP K368R vs. control cells were stimulated with 10 μg/ml PLA-LDL for 10 min. Levels of phospho-Akt, Akt and PKCα-EGFP K368R were determined by Western analysis. (B) Densitometric analysis of Western data showing the ratio of phospho-Akt vs. Akt. (C) THP-1 cells were transiently transfected with the PKCβ-EGFP kinase dead mutant K371M as described under Materials and Methods. Cells expressing PKCβ-EGFP K371M vs. control cells were stimulated with 10 μg/ml PLA-LDL for 10 min. Levels of phospho-Akt, Akt and PKCβ-EGFP K371M were determined by Western analysis. (D) Densitometric analysis of Western data showing the ratio of phospho-Akt vs. Akt. Data are presented as mean±SEM, n=3. *, P<0.05 vs. PLA-LDL.
LDL+H2O2.

with 1 mM H2O2 and PLA-LDL (10 μg/ml) for 8 h. (A) Cell viability based on Annexin V/PI staining or (B) loss of mitochondrial membrane potential was analyzed as described under Material and Methods. Data are presented as mean values±SEM, *P<0.05 vs. H2O2; **P<0.05 vs. PLA-LDL+H2O2.

as well as PI positive cells and 35% DiOC6 negative cells (Fig. 5). These markers of cell death were significantly attenuated when H2O2 was co-incubated with PLA-LDL. Protection elicited by PLA-LDL was lost when classical PKCs were blocked with Gö6976. These results imply that signalling of PLA-LDL via PKC is required for cell protection against H2O2-induced cell death.

4. Discussion

PLA-LDL induces different responses in monocytic cells, but signalling pathways underlying these actions remain obscure. Here, we present data suggesting a critical role of classical PKCs such as PKCα and PKCβ in activation of Akt and pro-survival signalling elicited by PLA-LDL in THP-1 monocytes. From our initial studies employing pharmacological inhibitors of PLC as well as intracellular calcium chelators we concluded that the formation of second messengers, known to activate classical PKC isoforms, is a pre-requisite for Akt phosphorylation by PLA-LDL. The involvement of classical PKC isoforms in signalling to Akt was confirmed by using Gö6976, which is considered to be specific for classical PKCs, at least at the concentrations used [21]. In addition, PKC depletion in response to TPA treatment eliminated Akt phosphorylation in response to PLA-LDL, providing further evidence towards the involvement of TPA-responsive PKC isoforms in PLA-LDL-induced signalling to Akt.

Regarding classical PKC isoforms, only PKCα and -β were detected in THP-1 cells [22]. PKCα and PKCβ were previously shown to participate in regulation of Akt [23,24]. Using two different molecular approaches to modulate the activity of PKCα and -β, we provide evidence that both classical isoforms present in THP-1 are involved in Akt activation by PLA-LDL.

Silencing PKCα expression by PKCα-specific siRNA reduced PKCα protein amount by more than 50%, which down-regulated PLA-LDL-stimulated Akt phosphorylation to a similar extent. Additionally, expression of a dominant negative PKCα-EGFP K368R mutant eliminated activation of Akt by PLA-LDL. Expression of dominant negative PKCβ-EGFP K371M mutant also suppressed PLA-LDL-induced Akt phosphorylation. Surprisingly, knockdown of PKCβ with specific siRNA resulted in concomitant PKCα down-regulation. This was unrelated to off-target effect of PKCβ siRNA since PKCα protein levels were unaffected by PKCβ siRNA. In contrast, expression of dominant negative PKCβ protein did not affect PKCα protein amount, suggesting that attenuating PKCβ activity does not affect PKCα expression.

It is thus reasonable that both, PKCα and PKCβ are involved in PLA-LDL-mediated Akt phosphorylation. Recently, a PKCβ-dependent activation pathway for PKCα was demonstrated [20]. However, the molecular mechanisms by which PKCα and PKCβ interact and modulate Akt phosphorylation remain unclear and deserve further analysis.

Data showing no impact of Gö6976 on translocation of the Akt-PH-EGFP construct in THP-1 cells suggest that PKC acts downstream of PI3K, most likely by directly modulating Akt S473 phosphorylation. The exact nature of the kinase phosphorylating Akt at S473, also known as PDK2, is still controversial. Several kinases, including PKCα and PKCβ, have been suggested to phosphorylate Akt at S473. Although both enzymes were shown to phosphorylate Akt in vitro [23], it is unclear whether its action in cells is direct or mediated by acting on other kinases such as Rictor/mTOR. Further studies should clarify the molecular target of classical PKCs in regulating Akt phosphorylation.

Activation of the PI3K-Akt pathway is associated with pro-survival response under a variety of physiological settings. We show that Gö6976 blocks a pro-survival response in THP-1 cells towards PLA-LDL, most likely by interfering with PKCα and PKCβ activation. The pro-survival role of both isoenzymes is supported by data pointing to an antiapoptotic effect of PKCα and -β in various cell types.
[25–29]. Under conditions of atherosclerosis progression, monocyte survival is considered to be atherogenic [3]. Thus, signalling mediated by PKCα and PKCβ in monocytes should have pro-atherogenic effect, not only by acting pro-survival but also considering the role of both isoenzymes in phagocyte superoxide production, another pro-atherogenic event [30–32]. This adds to the spectre of proatherosclerotic actions ascribed to PKC isoenzymes in diabetes and insulin resistance (reviewed in [15]). Further studies involving transgenic mouse models may help to clarify a possible role of PKCα and -β in the development of atherosclerosis.

Previously, PKC was associated with proliferative signals induced by oxidized LDL in macrophages. Although mechanistically unknown, it has been suggested that lysophosphatidylcholine evokes mitogenic actions of oxidized LDL [16,17]. Our data using PLA-LDL imply that non-esterified fatty acids (NEFA) are likely mediators of Akt activation in monocytes [4]. Therefore, the mechanism involving PKC activation in PLA-LDL-induced pro-survival response apparently differs from those pathways associated with proliferative signalling by oxidized LDL. Whether NEFA released from LDL by phospholipolysis directly activate PKCα [33] or use a receptor-mediated pathway is still unclear. However, based on the observation that a phosphoinositide-specific PLC is necessary for Akt activation makes a receptor pathway more likely. It was observed that PLA-LDL activates cytosolic PLA2 in mononuclear cells, which may contribute to PKC activation [34]. Surprisingly, we were unable to detect membrane translocation of PKCα and -β upon PLA-LDL addition (data not shown), suggesting that activation of only a minor fraction of PKCα or -β may be sufficient for Akt phosphorylation [35]. It was shown that in endothelial cells PKCα activation in lipid rafts may take place where Akt is also co-localized [23]. Whether this localized activation occurs in monocytes remains to be clarified.

In conclusion, a combination of pharmacological and molecular approaches identified a central role of the classical PKCα and PKCβ isoforms in PLA-LDL-induced Akt phosphorylation and initiation of a pro-survival signalling pathway in mononuclear cells. We suggest that phosphorylation of Akt S473 by classical PKCs occurs either directly or indirectly by modulating the activity of PDK2.

Acknowledgements

We thank Dr. Peter Downes for providing the Akt-PH-EGFP plasmid, Dr. Dominique Joubert for providing the PKCβ-EGFP K371M plasmid and Franz-Josef Streber for technical assistance. This study was supported by a grant from Deutsche Forschungsgemeinschaft (BR999).

References


