Pharmacological Reports
2011, 63, 19–29
ISSN 1734-1140

Review

Protein kinase Cε as a cancer marker and target for anticancer therapy

Ewa Totoń[1], Ewa Ignatowicz[2], Karolina Skrzeczkowska[1], Maria Rybczyńska[1]

[1]Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences, Przybyszewskiego 49, PL 60-355 Poznań, Poland

[2]Department of Pharmaceutical Biochemistry, Poznan University of Medical Sciences, Święcickiego 4, PL 60-781 Poznań, Poland

Correspondence: Maria Rybczyńska, e-mail: mrybczyn@ump.edu.pl

Abstract:
Protein kinase Cε (PKCε) is a representative member of a family of novel PKC isoforms that are independent of calcium, but can be activated by phorbol esters, diacylglycerol (DAG) and phosphatidylserine (PS). This kinase is capable of modulating crucial cell functions, including proliferation, differentiation and survival. These activities depend on enzyme translocation to subcellular compartments upon binding DAG, PS or exogenous stimulators. PKCε initiates malignant transformation of cells through its effects on the Ras/Raf/MAPK pathway and displays the greatest carcinogenic potential of all PKC isoforms. PKCε also promotes tumor metastatic capacity and resistance to anti-cancer therapy. Overexpression of PKCε is found in numerous cancers including colon, breast, stomach, prostate, thyroid and lung and is considered an important marker of negative disease outcome. Although overexpression of PKCε is observed in tumors, it is not found in healthy tissues hence it has been suggested as a diagnostic marker or a putative target for specific inhibitors used for treatment of cancer. Research on selective inhibition of PKCε is under way and diverse approaches may become clinically applicable anti-tumor strategies. Suppression of the PKCε-encoding gene achieved through the antisense cDNA, suppression of PKCε with RNAi and inhibition achieved with translocation-inhibitory peptides may provide novel treatment strategies for cancer.

Key words:
protein kinase Cε, carcinogenesis, protein kinase Cε inhibition

Abbreviations: AA – arachidonic acid, ATP – adenosine-5'-triphosphate, CG-NAP – centrosome and Golgi localized protein kinase N-associated protein, DAG – diacylglycerol, EGFR – epidermal growth factor receptor, PKA – protein kinase A, PKC – protein kinase C, PKN – protein kinase N, PMA – phorbol 12-myristate 13-acetate, PP1 – protein phosphatase 1, PP2A – protein phosphatase 2A, RACK(s) – receptor(s) for activated C-kinase, TNF – tumor necrosis factor, TRAIL – TNF-related apoptosis-inducing ligand

Introduction

Protein kinases became an area of interest in pharmacology when a growing body of evidence demonstrated their critical role in physiology and pathology. Over 500 kinases have been discovered in humans and their interplay in cellular metabolism and signal

transduction was termed the "kinome" in 2002. These kinases are encoded by 518 genes which represent 1.7% of the human genome [55]. Kinases catalyze the transfer of phosphate from an ATP molecule onto the hydroxyl moieties of serine, threonine or tyrosine in target proteins. Protein kinases are crucial signaling molecules in regulating cellular growth, differentiation, proliferation and death, embryogenesis, angiogenesis and immunological responses. Disregulation of protein kinase functions cause diverse pathologies, such as cancer [46], diabetes [77], stroke [16] and Alzheimer's disease [87].

The discovery of protein kinase C (PKC) was reported by Nishizuka et al. [67], who described an enzyme that was independent of cAMP but regulated by phospholipids and calcium. The protein kinase C family comprises at least 11 different isozymes, categorized into subgroups based on structural similarities and the ligands required for optimal activation. Conventional or classical PKCs (α, βI, βII and χ) are stimulated by diacylglycerol (DAG), phospholipids, phorbol esters and calcium; novel PKCs (δ, ε, η, θ and μ) are activated by DAG and phorbol esters but do not require calcium for functioning and atypical PKCs (ζ and ι) are insensitive to both DAG and phorbol esters and are calcium independent [33, 37, 42].

Various PKC isozymes are present in the same cell and mediate specific functions. The multiple activities of PKC in signal transduction are modulated by targeting the enzymes to specific intracellular compartments. Several PKC isozymes may be capable of phosphorylating the same protein substrate [88]. Ascribing individual PKC isozymes to specific cellular functions is restricted by difficulties in developing isozyme-selective agonists and/or antagonists [79]. The three subgroups of PKC isozymes are activated by different mechanisms, and activated PKCs undergo inactivation *via* phosphatase activity and the ubiquitin-proteasome pathway [7]. Both activation and inactivation of PKCs are regulated temporally and spatially [88].

PKCε structure and subcellular localization

The PKCε gene, *PRKCE*, has been mapped to human chromosome 2p21 [15]. PKCε is found in diverse tissues including neuronal, hormonal, heart, immune, retinal, endothelial and epidermal cells [6]. PKCε is a linear protein molecule comprising 737 amino acids. In the molecule two functional domains can be distinguished: a C-terminal kinase catalytic region, which is responsible for protein phosphorylation, and an N-terminal regulatory element that binds endogenous or exogenous stimulators, such as phorbol esters. These two domains are linked by a hinge region (Fig. 1). In the structure of the regulatory moiety of PKCε

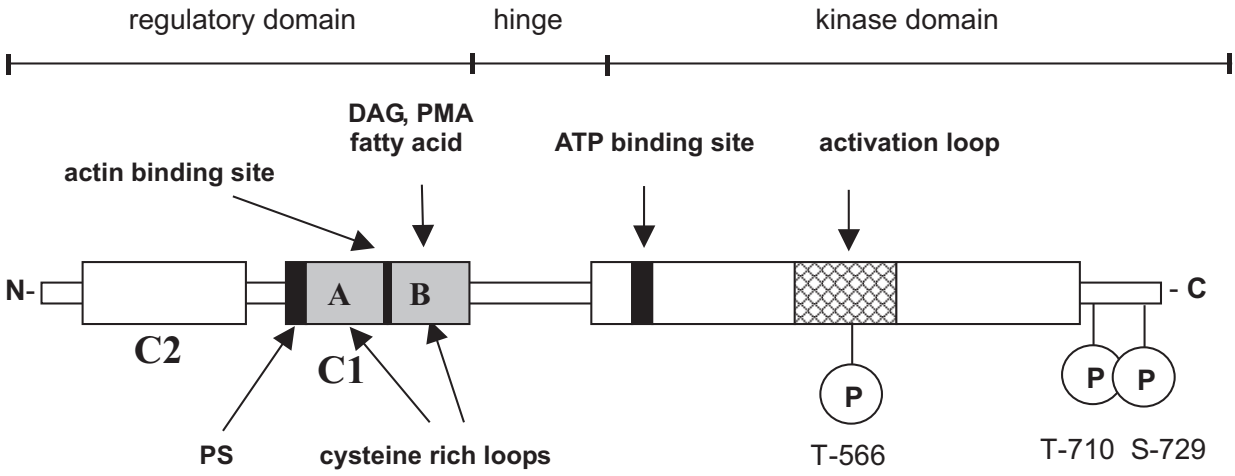

Fig. 1. Structure of PKCε. N – amino-terminal regulatory domain, C – carboxyl-terminal kinase catalytic domain, PS – pseudosubstrate motif, C1, C2 – membrane binding domains, DAG – diacylglycerol, PMA – phorbol 12-myristate 13-acetate, P – phosphate group, T – threonine, S – serine [3]

there is a variable region 1 (V1), a pseudosubstrate or autoinhibitory domain and membrane binding modules, called C1 and C2. The C1 domain is a target for activators, including phospholipids, DAG, or phorbol esters, which mimic DAG [44, 85]. In the resting state, PKCε stays inactive due to the binding of the pseudosubstrate motif to the catalytic site [85]. Association of activators with the regulatory domain changes the protein conformation so that the autoinhibitory pseudosubstrate module can be removed from the enzyme active site [66].

Region C1 contains two 50-amino acid loops rich in cysteine, called C1A and C1B, in which cysteine and histidine residues are arranged in a zinc finger motif. The C1B loop expresses stronger affinity to phorbol esters than C1A [40]. Domain C1 is involved in protein-protein interactions due to the unique actin-binding sequence (amino acids 223–228) situated between C1A and C1B [25, 44, 100], and it is essential for the effects of the kinase on cellular morphology. Actin filaments anchor PKCε, promote its recruitment to membranes and convert the kinase to the catalytically active conformation [3, 66]. Targeting of PKCε to specific cellular compartments depends on the type of second messenger and specific adapter proteins involved [3]. According to data presented by Kashiwagi et al. [44], region C1B determines different patterns of PKCε translocation upon activation by diverse lipid second messengers: arachidonic acid (AA) or ceramide direct PKCε to the Golgi, and phorbol esters cooperate with either C1A or C1B. Due to the high conformational flexibility of the C1 domain of PKCε, its translocation to various cellular compartments is faster than other PKCs; moreover, the open C1 domain allows PKCε to be activated by reactive oxygen species and other oxidative signals even in the absence of DAG [6].

The C2 domain of PKCε lacks the acidic residues that are essential for calcium coordination thus making the enzyme insensitive to calcium activation [98]. This domain mediates binding of activated PKCε to its specific receptors, the RACKs [18, 62]. Activation of PKCε and other novel PKCs by DAG or phorbol esters results in its translocation from the cytosol to membranous sites [67]. At present, there is little evidence demonstrating which protein is a preferred substrate for various PKCs, and several PKC isozymes may be capable of phosphorylating the same protein however, it is the regulatory domain structure that determines interaction partners and subcellular localization [42, 99].

In NIH 3T3 and 3T6 fibroblasts, glial cells (C6) and in uterine cancer (HeLaPKCεA/E), PKCε protein was found in two isoforms, with molecular weights 87 kDa and 95 kDa ($PKC\varepsilon^{87}$ and $PKC\varepsilon^{95}$). Isoform $PKC\varepsilon^{95}$ is characteristic of confluent cells while $PKC\varepsilon^{87}$ is the predominant form in adherent cells [25, 29]. NIH3T3 fibroblasts represent a cell line extensively studied for the reactivity of various PKC isoforms. In the cytoplasm of inactive cells, PKCε is present in the form of small granules resembling the endoplasmic reticulum [32]. Short-term activation with 12-O-tetradecanoylphorbol-13-acetate (PMA) directed PKCε mostly to the plasma membrane where it accumulated in the region of intercellular connections and to a lesser extent in the margins of cells [32, 61] and the Golgi network [45]. Stanwell et al. [83] demonstrated that the holo-enzyme was localized to the Golgi and other compartments, whereas the domain containing a zinc-finger motif was found exclusively at the Golgi. Results presented by Lehel et al. [50, 51] suggest that both the holo-enzyme and the zinc finger domain are capable of regulating Golgi-related processes, such as glycosaminoglycan sulfation. PKCε is localized mainly in the Golgi through its C1B domain, and a short-term treatment with PMA drives translocation to the plasma membrane [44, 45]. Our studies confirmed that in HeLaPKCεA/E cells, which express constitutively active PKCε, the kinase accumulated mainly in the Golgi, and upon short-term treatment with PMA the enzyme was found primarily in the cell membrane [29].

PKCε phosphorylation

Reversible phosphorylation of PKCε changes its molecular conformation and activity and affects its capacity to react to various stimulants. For an optimal response to second signal messengers, which is dependent upon C1 and C2 domains, PKCε requires phosphorylation of three aminoacids in the protein core: threonine 566 (Thr-566, T-566) in the activation loop, threonine 710 (Thr-710, T-710) at the autophosphorylation site and serine 729 (Ser-729, S-729) in the C-terminal hydrophobic region [13, 96]. Thr-566 is phosphorylated by the phosphoinositide-dependent kinase-1 (PDK1). As PDK1 expresses constant activity, it is believed that this process is regulated by the

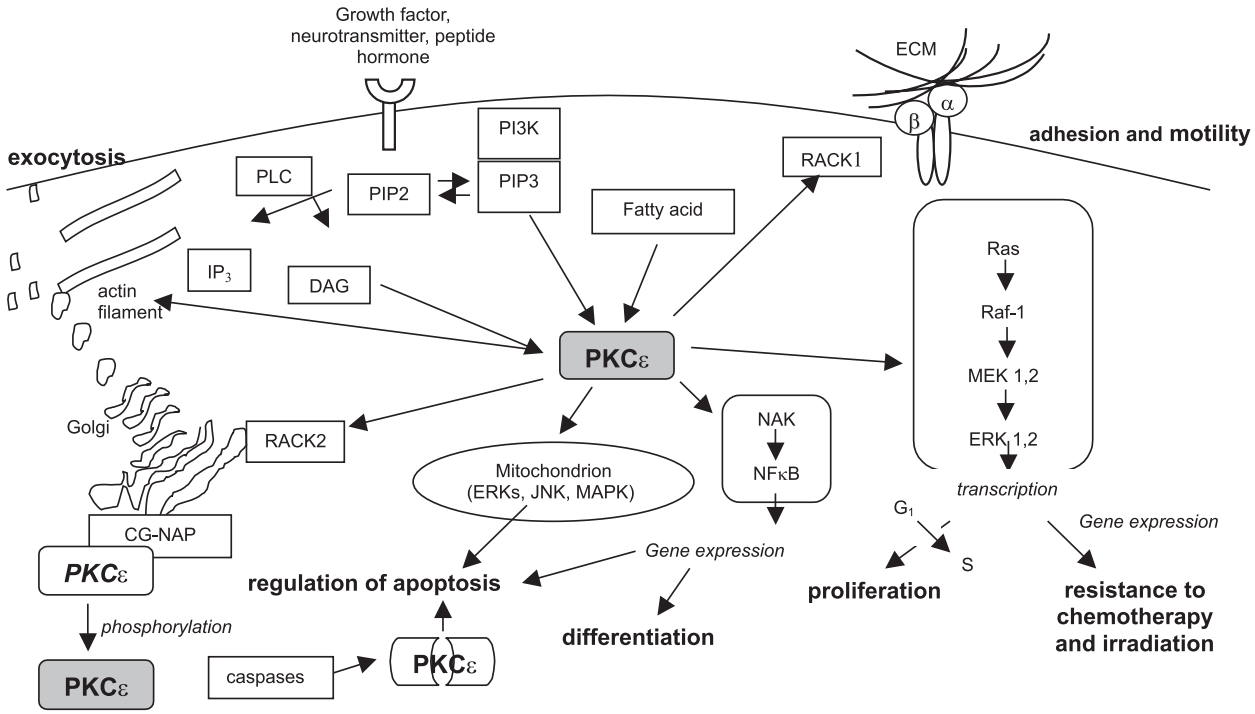

Fig. 2. Activation and role of PKCε in cells. ECM – extracellular matrix, α-β – integrin, CG-NAP – centrosome and Golgi localized PKN associated protein, DAG – diacylglycerol, IP_3 – inositol 3,4,5-trisphosphate, PI3K – phosphoinositide 3-kinase, PIP_3 – phosphatidylinositol 3,4,5-trisphosphate, PLC – phospholipase C, RACK1 – receptor for activated C kinase, Raf-1 – serine threonine-protein kinase, Ras – proteins involved in cellular signal transduction, ERK – extracellular signal-regulated kinases, JNK – c-Jun N-terminal kinase, originally identified as the kinase that binds and phosphorylates c-Jun kinase, MAPK – mitogen-activated protein (MAP) kinase [3]

modulation of substrate conformation and localization. Non-phosphorylated PKCε or minimally phosphorylated PKCε (immature form) binds the endogenous anchoring protein CG-NAP through its catalytic domain. CG-NAP is a common adaptor for protein kinases N (PKN) and A (PKA) and protein phosphatases PP1 and PP2A which are found in the Golgi and centrosome [3]. Following PKCε binding to CG-NAP, the autophosphorylation of Thr-710 and Ser-729 likely occurs. However, the possibility that Ser-729 is directly phosphorylated by PKN, which modulates the PDK1 activity in concert with CG-NAP, cannot be excluded. Lastly, the fully phosphorylated PKCε dissociates from CG-NAP and is ready to respond to second messengers demonstrating that CG-NAP is indispensable for effective enzyme phosphorylation [96, 97].

Mass spectroscopy studies have revealed that the $PKC\varepsilon^{95}$ isoform undergoes phosphorylation at Thr-566, Ser-703 and Ser-729, whereas $PKC\varepsilon^{87}$ is phosphorylated only at Thr-566 and Ser-703. It is believed that phosphorylation at Ser-729 is essential for PKCε translocation within the cell [24].

Role of PKCε in cellular metabolism and carcinogenesis

Numerous cellular activities are modulated by PKCε interaction with cytoskeletal structures, including migration and adhesion [4, 8, 17] (Fig. 2), proliferation [5], and differentiation [74]. Gene expression [26], transport mechanisms [43, 59], inflammation and immunity and secretory functions [3], and muscle mechanical force adaptation [56, 89] are also affected by PKCε-mediated mechanisms. Due to the actin-binding motif present in the C1 regulatory domain of PKCε it is able to regulate cell growth and morphology: e.g., in a homeostatic nervous system, PKCε induces axon formation during stem cell differentiation into neurons [100].

The confined activation of PKCε plays a protective role in cardiac ischemia and the Alzheimer's disease development; however, chronic PKCε activation may also lead to hyperglycemia and diabetes [3, 43]. Increased levels of PKCε in muscles suppresses the ac-

tivity of the insulin receptor causing a subsequent rise in lipid storage in cells, glycogen synthesis and insulin resistance [38], which results in the development of cardiomyopathy and nephropathy [3].

In their early experiments Mischak et al. [61] showed that overexpression of PKCε induces anchorage-independent cellular growth in cells not exposed to any stimulant. Moreover, cells overexpressing PKCε form tumors in nude mice with an incidence rate of 100%. PKCε shows the greatest oncogenic potential of the PKC family [3, 11, 37, 96] and numerous reports associate excessive PKCε activation with tumor promotion [7, 36, 37, 70] and apoptosis suppression [19, 27, 54, 57, 68]. Overexpression of PKCε was found in cancers of the colon [3], breast [63], stomach [81], prostate [94], thyroid, lung [37], aggressive gliomas [68] and hairy cell leukemia [80]. In tumors grown *in vivo*, binding of actin to the C1 domain of PKCε is responsible for invasion and metastasis [4]. In neuroblastoma cells (SK-N-BE) axon growth can be prevented by the constitutive kinase inhibitor, ceramide C2 [78].

Contribution of PKCε to carcinogenesis depends on the Ras/Raf/MAP kinase cascade, which is a well-examined pathway of intracellular signal transmission. Activation of the Ras/Raf/MAP kinase cascade results in the transcription of genes involved in cell proliferation and growth. Impairment of this complex regulatory system promotes malignant transformation, invasiveness and metastasis and suppresses apoptosis of cancer cells [7]. Stimulation of the Ras protein results in translocation of Raf-1 kinase from the cytosolic compartment to the plasma membrane where Raf-1 is activated through a set of specific kinases including PKCε. Results from studies on the involvement of the Ras/Raf/MAPK pathway in modulation of oncogenic function of PKCε suggest that PKCε interacts with Ras signaling downstream of Ras but upstream of Raf-1 [7]. Two different theories have been proposed to describe PKCε involvement in Raf-1 activation: either PKCε directly phosphorylates Raf-1 protein [35] or the whole process is indirectly modulated by growth factor secretion [12, 90]. Overexpression of PKCε was shown to increase Akt (a family of serine/threonine kinases) protein levels and activation, which is essential for the anti-apoptotic effects of numerous Akt phosphorylated substrates, including caspase-9 and Forkhead transcription factors. Also, an indirect regulation of Akt by PKCε was observed through interactions with integrins and/or the secretion of growth factors [7]. These and other studies *in vitro* and *in vivo* confirm that both Akt and PKCε collaborate to promote cell survival; however, some reports suggest that the survival-promoting effects of PKCε depend on the type of stimulus and cell type and may be unrelated to Akt activation [7].

Anti-apoptotic ability of PKCε also depends on the increased expression of the anti-apoptotic proteins of the Bcl-2 family and suppression of the pro-apoptotic members of the Bcl-2 family [7]. Additionally, overexpression of the constitutively activated PKCε increases activation of nucleophosmine (NPM), a phosphoprotein capable of inducing carcinogenesis [47, 48]. In human cancer cells overexpression of the *NPM* gene inhibits apoptosis caused by the UV light or hypoxia [49, 52]. Results of our studies show correlation between the PKCε expression and NPM activity in HeLaPKCε A/E cells [29].

PKCε also plays an important role in cancer invasiveness and metastasis which was first reported on by Chun et al. in 1996 [17]. They examined six PKC isoforms (α, γ, ε, λ, ζ, ι) present in uterine cancer cells (HeLa) and found that only PKCε undergoes translocation in the process of adhesion to the gelatin matrix. Perletti et al. [73] observed spontaneous extension of long membrane protrusions in NIH3T3 fibroblasts which overproduce PKCε. These changes in cellular morphology are similar to those acquired in the oncogene ras-transformed fibroblasts. Formation of the elongated structures depended on an actin-binding motif in the regulatory domain C1, which also promoted an increase in the activity of pericellular metalloproteinase, invasion of the Matrigel barrier *in vitro* and the invasion and metastasis of tumors grown *in vivo* in nude mice upon inoculation with NIH3T3 fibroblasts overexpressing PKCε [4]. Studies on cooperation of PKCε with actin and keratin showed that invasiveness and metastasis are affected by PKCε-mediated phosphorylation of cytoskeletal proteins triggering detachment and migration of cancer cells [4]. Additionally, PKCε was shown to induce hypoxia, thus promoting cancer progression and conferring resistance to radio- and chemotherapy [81].

In the murine model of carcinogenesis, overexpression of PKCε in the epidermis caused a rise in cytokine production and release, which impaired hematopoiesis in bone marrow, disturbed normal proportions of neutrophils and eosinophils and led to myeloproliferative-like disease [92]. Investigation of human squamous cell carcinoma revealed that high expres-

sion of PKCε in epidermal tissue might create a microenvironment that enhances cancer development by a paracrine mechanism [93].

Recently, Dittmann et al. [21] described the role of PKCε in phosphorylation of the epidermal growth factor receptor (EGFR), which is a key step in translocation of EGFR to the nucleus, where it induces the transcription of genes essential for cell survival after stress exposure (e.g., irradiation). In cancer cells, activation of EGFR-mediated signaling leads to a decrease in radiosensitization and an increase in DNA repair.

PKCε as a molecular target in the anticancer therapies

Research examining the molecular basis of carcinogenesis and other diseases, and the subsequent investigation of pharmacological approaches to treat these diseases, has drawn attention to PKCE as a pharmacological target for the treatment of various diseases.

Inhibitors of classical protein kinases C have been investigated for their therapeutic applications for approximately 20 years and novel isozymes have recently become a matter of interest to fundamental research and the pharmaceutical industry. The localization of PKCε in numerous tissues and its importance in key cellular pathways raises the possibility of many therapeutic strategies based on the inhibition of this isozyme; however, activation of PKCε has been shown to exert tissue-protective effects [37, 88].

PKCε was found to provide protection in the heart during ischemia/reperfusion-induced damage [22]. Short-term periods of cardiac ischemia protected heart from the prolonged episodes of ischemia and selective activating of PKCε either directly or indirectly would mediate this preconditioning. In ischemic tissue, mitochondrial aldehyde dehydrogenase 2 (ALD2) is a direct substrate of PKCε, and modulation of ALD2 may decrease formation of cytotoxic aldehydes and thus reduce ischemic damage [14]. These effects are particularly necessary during cardiac surgery with extracorporeal circulation or prior to cardiac transplant surgery [39]. In ischemic cerebral tissue, a similar protective effect of moderate PKCε activation was found and application of ψεRACK, a PKCε-specific activator peptide conferred tolerance to cerebral ischemic reperfusion injury [10]. Additionally, newly discovered PKCε activators developed through cyclopropanation of polyunsaturated fatty acids led to the reduction of β-amyloid in neuronal cells and might be useful for the treatment of Alzheimer's disease [65].

Studies on immune system function following infection showed that PKCε is a target for bacterial lipopolysaccharide signals, which leads to NF-κB and MAPK activation, lymphocyte T suppression and inflammation development. Selective inhibition of PKCε either by peptides or antisense oligonucleotides could provide a therapeutic approach for the treatment of certain T-lymphocyte dependent inflammatory diseases [1].

Extensive research in the past several years has brought about the discovery of a few short peptides (7–12 amino acids long) capable of PKC isozyme-selective inhibition. These peptides selectively hamper the interaction between activated kinases and their specific anchoring proteins, RACKs, and may have potential pharmacological applications [2, 82]. Another set of short peptides (6–8 amino acids long) represent translocation-specific agonists due to their homology to sequences within their respective RACK (εRACK). Single amino acid substitution was shown to convert a PKCε-selective agonist into an antagonist [53]. The PKCε antagonist binds to the RACK-binding site within the PKCε molecule, whereas the previously discovered peptide inhibitor, εV1-2, which is derived from the C2 domain, is able to bind εRACK [53].

Diverse mechanisms lead to PKCε inhibition though involvement of different cellular partners. It may offer the possibility of synergy if PKCε-inhibitory peptides (e.g., εV1-2, ψRACK) find therapeutic application [53]. Translocation-inhibitory peptide εV1-2 was submitted to clinical Phase IIa trials for patients with postoperative pain [22, 98].

PKCε is centrally involved in driving cells to malignant transformation and might be a diagnostic marker of tumorigenesis as there have been no reports showing increased activity of PKCε in normal tissues [76].

PKCε may also be a therapeutic target for the early stages of cancer or in suppressing cancer invasion and metastasis. Studies on other PKC isozymes have shown promising findings with respect to selective inhibition of PKC isozymes and other therapeutic applications. At present, transcriptional suppression of PKCα with antisense oligonucleotides was shown to exert antitumor activity in lung cancer patients. Early clinical studies showed promising results for the antisense oligonucleotide, LY900003, applied in combination with cisplatin and gemcitabine [91]; however,

Phase III clinical trial did not confirm the superiority of this novel therapy over the former therapeutic regimes in non-small cell lung carcinoma patients [88, 98].

Studies on resistance to anticancer drugs showed that this undesirable phenomenon is correlated with the PKCε protein levels [20, 28]. Ding et al. [20] demonstrated that resistance or susceptibility of human lung cancer cells to chemotherapeutic agent(s) depends on the level of *PRKCE* gene expression. Overexpression of the gene enhances the resistance due to the caspases inhibition, which promotes cancer cell survival, while suppression of the *PRKCE* gene achieved through the antisense cDNA, increases chemotherapeutic effect. Further research confirmed the finding of the anti-apoptotic role of PKCε overexpression in cancer cells [30, 54, 68]. These functions are exerted by affecting activation and/or concentration of Bcl-2 proteins, which influence the integrity of mitochondria. PKCε alters the proportions between pro- and anti-apoptotic proteins in Bcl-2 family [7].

Apoptosis induced by tumor necrosis factor (TNF-α) in MCF-7 breast cancer cells was decreased by PKCε overexpression, which inhibited Bax translocation to the mitochondria [54]. In HL-60 cells exposed to peptide εV1-2, a specific inhibitor of PKCε, the anti-apoptotic potential of PKCε was blocked. Further treatment with TRAIL (TNF-related apoptosis-inducing ligand) caused cell apoptosis [31]. Also, in prostate cancer cells (LNCaP), PKCε was suggested to relate inversely with Bax, a pro-apoptotic protein member of Bcl-2 family, and inhibition of mitochondrial-dependent apoptotic signaling [58]. Mayne et al. [57] showed that phorbol esters protected leukemia cells (U937) against apoptosis induced with TNF-α and calphostin C. Inhibition of PKCε with εV1-2 reversed this protection and re-induced apoptosis.

Inhibition of PKCε expression achieved in breast cancer cells (MDA-MB231) after exposure to RNAi fragments caused a significant decrease in proliferation and tumor growth [71]. The head and neck squamous cell cancer reacted likewise: suppression of PKCε with RNAi resulted in invasiveness and motility reduction in cancer cells [72]. Additionally, Stensman et al. [86] observed that in the neuroblastoma cells (SK-N-BE2C), in which motility was increased upon treatment with phorbol esters, application of PKCε siRNA reduced PKCε activity and cell invasiveness.

It is thought that the molecular mechanisms of PKCε-regulated inhibition of motility and adhesion depend on PKCε binding to β1 integrin and F-actin. PKCε affects adhesion, spread and motility of human glial cancer through its influence on focal adhesion, lamellopodia formation and integrin accumulation [8, 84]. In breast cancer cells, fatty acids stimulate β1 integrin-dependent adhesion to type IV collagen through activation of protein kinases ε and μ [69]. Studies by Brenner et al. [9] in breast cancer cells (CCF-RC1) showed PKCε involvement in the regulation of β1 integrin expression. Suppression of PKCε activity with PKC inhibitor, Ro-31-8220 resulted in subsequent reduction of β1 integrin expression. Intracellular integrin circulation and recycling modulation by PKCε was demonstrated by Ivaska et al. [41]; they showed that PKC co-localized with β1 integrin in plasma membrane vesicles. Release of PKCε from these structures depended not only on energy (cellular reserves of ATP and GTP) but also on the catalytic activity of the enzyme. Sustained activation of β1 integrins induced survival of recurrent prostate cancer cells (CWR-R1), which probably contributes to the failure of hormonal therapy [95]. Involvement of integrins in tumor progression drove attention to possible suppressing of these proteins as anticancer/antiangiogenic strategy. These goals can be achieved through use of monoclonal antibodies; the monoclonal antibody volociximab is currently in the Phase II clinical trials [75].

In conclusion, it has been extensively shown that PKC plays a critical role in various signaling systems essential for physiology and pathology. Protein kinases inhibitors represent an important group of new anticancer drugs, and due to its pleiotropic involvement in cell growth, proliferation and survival, PKCε is an appealing subject for molecular biology and biotechnology research. The number of patent applications for specific kinase inhibitors is constantly raising, although results of clinical trials do not always meet our expectations. Success in the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors demonstrated that inhibition of a single oncogenic kinase provides desirable effects [23, 34]; however, attempts to affect cancer angiogenesis did not show comparably positive results [64]. The least promising effects of kinase inhibitors were found in the treatment of breast, lung, colon, prostate and pancreas cancers; however, the research of Liron et al., [53] directed attention to various targets of PKC and corresponding molecular partners. Discovery of RNA interference made screening and selective knock-

down of genes involved in tumor proliferation possible. This technique may also provide tools for the attenuation of other cellular signaling pathways indirectly associated to carcinogenesis (e.g., NF-κB) [60].

Successful recognition of PKCε as a cancer marker and a target for selective drugs may only be a question of time, due to new discoveries in tumor biology and pharmaceutical advances. There is considerable hope for nanomaterials as efficacious carriers of apoptosis inducers and/or selective signal transduction blockers.

References:

1. Aksoy E, Goldman M, Willems F: Protein kinase C epsilon: a new target to control inflammation and immune-mediated disorders. Int J Biochem Cell Biol, 2004, 36, 183–188.
2. Aley KO, Messing RO, Mochly-Rosen D, Levine JD: Chronic hypersensitivity for inflammatory nociceptor sensitization mediated by the epsilon isozyme of protein kinase C. J Neurosci, 2000, 20, 4680–4685.
3. Akita Y: Protein kinase C-ε (PKC-ε): Its unique structure and function. J Biochem, 2002, 132, 847–852.
4. Akita Y: Protein kinase Cε multiple roles in the function of, and signaling mediated by, the cytoskeleton. FEBS J, 2008, 275, 3995–4004.
5. Balciunaite E, Jones S, Toker A, Kazlauskas A: PDGF initiates two distinct phases of protein kinase C activity that make unequal contributions to the G0 to S transition. Curr Biol, 2002, 10, 261–267.
6. Barnett ME, Madgwick DK, Takemoto DJ: Protein kinase C as a stress sensor. Cell Signal, 2007, 19, 1820–1829.
7. Basu A, Sivaprasad U: Protein kinase Cε makes the life and death decision. Cell Signal, 2007, 19, 1633–1642.
8. Besson A, Wilson TL, Young WV: The anchoring protein RACK1 links protein kinase Cε to integrin β chains. J Biol Chem, 2002, 277, 22073–22084.
9. Brenner W, Benzing F, Gudejko-Thiel J, Fischer R, Farber G, Hengstler JG, Seliger B, Thüroff JW: Regulation of beta1 integrin expression by PKCepsilon in renal cancer cells. Int J Oncol, 2004, 25, 1157–1163.
10. Bright R, Sun GH, Yenari MA, Steinberg GK, Mochly-Rosen D: εPKC confers acute tolerance to cerebral ischemic reperfusion injury. Neurosci Lett, 2008, 441, 120–124.
11. Cacace AM, Guadagno SN, Krauss RS, Fabbro D, Weinstein IB: The epsilon isoform of protein kinase C is an oncogene when overexpressed in rat fibroblasts. Oncogene, 1993, 8, 2095–2104.
12. Cai H, Smola U, Wixler V, Eisenmann-Tappe I, Diaz-Meco MT, Moscat J, Rapp U, Cooper GM: Role of diacylglycerol-regulated protein kinase C isotypes in growth factor activation of the Raf-1 protein kinase. Mol Cell Biol, 1997, 17, 732–741.
13. Cenni V, Döppler H, Sonnenburg ED, Maraldi N, Newton AC, Toker A: Regulation of novel protein kinase Cε by phosphorylation. Biochem J, 2002, 363, 537–545.
14. Chen CH, Budas GR, Churchill EN, Disatnik MH, Hurley TD, Mochly-Rosen D: Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. Science, 2008, 321, 1493–1495.
15. Chen X, Knauf JA, Gonsky R, Wang M, Lai EH, Chissoe S, Fagin JA, Korenberg JR: From amplification to gene in thyroid cancer: a high-resolution mapped bacterial-artificial-chromosome resource for cancer chromosome aberrations guides gene discovery after comparative genome hybridization. Am J Hum Genet, 1998, 63, 625–637.
16. Chou WH, Messing RO: Hypertensive encephalopathy and the blood-brain barrier: is δPKC a gatekeeper? J Clin Invest, 2008, 118, 17–20.
17. Chun JS, Ha MJ, Jacobson BS: Differential translocation for protein kinase C ε during HeLa cell adhesion to a gelatin substratum. J Biol Chem, 1996, 271, 13008–13012.
18. Csukai M, Chen CH, De Matteis MA, Mochly-Rosen D: The coatomer protein β'-COP, a selective binding protein (RACK) for protein kinase Cε. J Biol Chem, 1997, 272, 29200–29206.
19. De Gruij LF: Protein kinase Cε reveals importance of extrinsic apoptosis in preventing UV carcinogenesis. J Invest Dermatol, 2009, 129, 1853–1856.
20. Ding L, Wang H, Lang W, Xiao L: Protein Kinase C-ε promotes survival of lung cancer cells by suppressing apoptosis through dysregulation of the mitochondrial caspase pathway. J Biol Chem, 2002, 277, 35305–35313.
21. Dittman K, Mayer C, Rodemann HP: Nuclear EGFR as novel therapeutic target. Strahlther Onkol, 2010, 1, 1–6.
22. Dorn GW II, Souroujon MC, Liron T, Chen CH, Gray MO, Zhou HZ, Csukai M et al.: Sustained in vivo cardiac protection by a rationally designed peptide that causes epsilon protein kinase C translocation. Proc Natl Acad Sci USA, 1999, 96, 12798–12803.
23. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB et al.: Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med, 2001, 344, 1031–1037.
24. England K, Rumsby MG: Changes in protein kinase Cε phosphorylation status and intracellural localization as 3T3 and 3T6 fibroblasts grow to confluency and quiescence: a role for phosphorylation at Ser-729? Biochem J, 2000, 352, 19–26.
25. England K, Watson J, Beale G, Warner M, Cross J, Rumsby M: Signalling pathways regulating the dephosphorylation of Ser729 in the hydrophobic domain of protein kinase C epsilon upon cell passage. J Biol Chem, 2001, 276, 10437–10442.
26. Eun DW, Ahn SH, You JS, Park JW, Lee EK, Lee HN, Kang GM et al.: PKCε is essential for gelsolin expression by histone deacetylase inhibitor apicidin in human cervix cancer cells. Biochem Biophys Res Commun, 2007, 354, 769–775.
27. Felber M, Sonnemann J, Beck JF: Inhibition of novel protein kinase Cε augments TRAIL-induced cell death in A549 lung cancer cells. Pathol Oncol Res, 2007, 13, 295–301.
28. Flescher E, Rotem R: Protein kinase C ε mediates the induction of P-glycoprotein in LNCaP prostate carcinoma cells. Cell Signal, 2002, 14, 37–43.

29. Garczarczyk D, Toton E, Biedermann V, Rosivatz E, Rechfeld F, Rybczynska M, Hofmann J: Signal transduction of constitutively active protein kinase C. Cell Signal, 2009, 21, 745–752.
30. Gillespie S, Zhang XD, Hersey P: Variable expression of protein kinase Cε in human melanoma cells regulates sensitivity to TRAIL-induced apoptosis. Mol Cancer Ther, 2005, 4, 668–676.
31. Gonalli A, Milani D, Rimondi E, Grill V, Celeghini C: Activation of PKC-ε counteracts maturation and apoptosis of HL-60 myeloid leukemic cells in response to TNF family members. Eur J Histochem, 2009, 23, 177–182.
32. Goodnight JA, Mischak H, Kolch W, Mushinski JF: Immunocytochemical localization of eight protein kinase C isozymes overexpressed in NIH 3T3 fibroblasts. J Biol Chem, 1995, 270, 9991–10001.
33. Gorin AM, Pan Q: Protein kinase Cε: an oncogene and emerging tumor biomarker. Mol Cancer, 2009, 8, 9.
34. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS et al.: Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol, 2003, 21, 4342–4349.
35. Hamilton M, Liao J, Cathcart MK, Wolfman A: Constitutive association of c-N-Ras with c-Raf-1 and protein kinase Cε in latent signaling modules. J Biol Chem, 2001, 276, 29079–29090.
36. Heidkamp MC, Ivengar R, Szotek EL, Cribbs LL, Samarel AM: Protein kinase Cepsilon-dependent MARCKS phosphorylation in neonatal and adult rat ventricular myocytes. J Mol Cell Cardiol, 2007, 42, 422–431.
37. Hofmann J: Protein kinase C isozymes as potential targets for anticancer therapy. Curr Cancer Drug Targets, 2004, 4, 1–11.
38. Ikeda Y, Olsen GS, Ziv E, Hansen LL, Busch AK, Hansen BF, Shafrir E, Mosthaf-Seedorf L: Cellular mechanism of nutritionally induced insulin resistance in Psammomys obesus: overexpression of protein kinase Cε in skeletal muscle precedes the onset of hyperinsulinemia and hyperglycemia. Diabetes, 2001, 50, 584–592.
39. Inagaki K, Churchill E, Mochly-Rosen D: Epsilon kinase C as a potential therapeutic target for the ischemic heart. Cardiovasc Res, 2006, 70, 222–230.
40. Irie K, Oie K, Nakahara A, Yanai Y, Oshigashi H, Wender PA, Fukuda H et al.: Molecular basis for protein kinase C isozyme-selective binding: the synthesis, folding, and phorbol ester binding of the cysteine-rich domains of all protein kinase C isozymes. J Am Chem Soc, 1998, 120, 9159–9167.
41. Ivaska J, Whelan RD, Watson R, Parker PJ: PKCε controls the traffic of β1 integrins in motile cells. EMBO J, 2002, 21, 3608–3619.
42. Jaken S: Protein kinase C isozymes and substrates, Curr Opin Cell Biol, 1996, 8, 168–173.
43. Kao YS, Fong JC: Endothelin-1 induction of Glut1 transcription in 3T3-L1 adipocytes involves distinct PKCε- and p42/p44 MAPK-dependent pathways. Biochim Biophys Acta, 2008, 1780, 154–159.
44. Kashiwagi K, Shirai Y, Kuriyama M, Sakai N, Saito N: Importance of C1B domain for lipid messenger-induced targeting of protein kinase C. J Biol Chem, 2002, 277, 18037–18045.
45. Kazi JU, Soh JW: Isoform-specific translocation of PKC isoforms in NIH3T3 cells by TPA. Biochem Biophys Res Commun, 2007, 364, 231–237.
46. Klein S, Levitzki A: Signal transduction therapy for cancer – whither now? Current Signal Transduction Therapy, 2006, 1, 1–12.
47. Kondo T, Minamino N, Nagamura-Inoue T, Matsumoto m, Taniguchi T, Tanaka N: Identification and characterization of nucleophosmin/B23/numatrin which binds the anti-oncogenic transcription factor IRF-1 and manifests oncogenic activity. Oncogene, 1997, 15, 1275–1281.
48. Korgaonkar C, Hagen J, Tompkins V, Frazier AA, Allamargot C, Quelle FW, Quelle DE: Nucleophosmin (B23) targets ARF to nucleoli and inhibits its function. Mol Cell Biol, 2005, 25, 1258–1271.
49. Kurki S, Peltonen K, Latonen L, Kiviharju TM, Ojala PM, Meek D, Lacho M: Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. Cancer Cell, 2004, 5, 465–475.
50. Lehel C, Olah Z, Jakab G, Anderson WB: Protein kinase Cε is localized to the Golgi via its zinc-finger domain and modulates Golgi function. Proc Natl Acad Sci USA, 1995, 92, 1406–1410.
51. Lehel C, Olah Z, Jakab G, Szallasi Z, Petrovics G, Harta G, Blumberg P: Protein kinase Cε subcellular licalization domains and proteolytic degradation sites. J Biol Chem, 1995, 270, 19651–19658.
52. Li J, Zhang X, Sejas DP, Bagby GC, Pang Q: Hypoxia-induced nucleophosmin protects cell death through inhibition of p53. J Biol Chem, 2004, 279, 41275–41279.
53. Liron T, Chen LE, Khaner H, Vallentin A, Mochly-Rosen D: Rational design of a selective antagonist of ε protein kinase C derived from the selective allosteric agonist, pseudo-RACK peptide. J Mol Cell Cardiol, 2007, 42, 835–841.
54. Lu D, Sivaprasad U, Huang J, Shanker E, Morrow S, Basu A: Protein kinase Cε protects MCF-7 cells from TNF-mediated cells death by inhibiting Bax translocation. Apoptosis, 2007, 12, 1893–1900.
55. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S: The protein kinase complement of the human genome. Science, 2002, 298, 1912–1934.
56. Mansour H, De Tombe PP, Samarel AM, Russel B: Restoration of resting sarcomere length after uniaxial static strain is regulated by protein kinase Cε and focal adhesion kinase. Circ Res, 2004, 94, 642–649.
57. Mayne GC, Murray AW: Evidence that protein kinase Cε mediates phorbol ester inhibition of calphostin C- and tumor necrosis factor-α-induced apoptosis in U937 histiocytic lymphoma cells. J Biol Chem, 1998, 273, 24115–24121.
58. McJilton MA, Van Sikes C, Wescott GG, Wu D, Foreman TL, Gregory CW, Weidner DA et al.: Protein kinase Cε interacts with Bax and promotes survival of human prostate cancer cells. Oncogene, 2003, 22, 7958–7968.
59. Mehta KD, Radominska-Pandya A, Kapoor GS, Dave B, Atkins BA: Critical role of diacylglycerol- and phospholipid-regulated protein kinase Cε in induction of low-density lipoprotein receptor transcription in response to

depletion of cholesterol. Mol Cell Biol, 2002, 22, 3783–3793.
60. Meylan E, Dooley AL, Feldser DM, Shen L, Turk E, Ouyang C, Jacks T**:** Requirement for NF-κB signalling in a mouse model of lung adenocarcinoma. Nature, 2009, 462, 104–107.
61. Mischak H, Goodnight JA, Kolch W, Martiny-Baron G, Schaechtle C, Kazanietz MG, Blumberg PM et al.: Overexpression of protein kinase C-δ and ε in NIH 3T3 cells induces opposite effects on growth, morphology, anchorage dependence and tumorigenicity. J Biol Chem, 1993, 268, 6090–6096.
62. Mochly-Rosen D: Localization of protein kinases by anchoring proteins: a theme in signal transduction. Science, 1995, 268, 247–251.
63. Morse-Gaudio M, Connolly JM, Rose DP: Protein kinase C and its isoforms in human breast cancer cells: relationship to the invasive phenotype. Int J Oncol, 1998, 12, 1349–1354.
64. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S et al.: Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med, 2007, 356, 115–124.
65. Nelson TJ, Cui C, Luo Y, Alkon DL: Reduction of β-amyloid levels by novel protein kinase Cε activators. J Biol Chem, 2009, 284, 34514–34521.
66. Newton AC: Regulation of protein kinase C. Curr Opin Cell Biol, 1997, 9, 161–167.
67. Nishizuka Y: The heterogeneity and differential expression of multiple species of the protein kinase C family. Biofactors, 1988, 1, 17–20.
68. Okhrimenko H, Lu W, Xiang C, Hamburger N, Kazimirsky G, Brodie C: Protein kinase C-ε regulates the apoptosis and survival of glioma cells. Cancer Res, 2005, 65, 7301–7309.
69. Palmantier R, George MD, Akiyama SK, Wolber FM, Olden K, Roberts JD: *cis*-Polyunsaturated fatty acids stimulate β_1 integrin-mediated adhesion of human breast carcinoma cells to type IV collagen by activating protein kinases C-ε and -μ. Cancer Res, 2001, 61, 2445–2452.
70. Palmantier R, Roberts JD, Glasgow WC, Eling T, Olden K: Regulation of the adhesion of a human breast carcinoma cell line to type IV collagen and vitronectin: roles for lipoxygenase and protein kinase C. Cancer Res, 1996, 56, 2206–2212.
71. Pan Q, Bao LW, Kleer CG, Sabel MS, Griffith KA, Teknos TN, Merajver SD: Protein kinase Cε is a predictive biomarker of aggressive breast cancer and a validated target for RNA interference anticancer therapy. Cancer Res, 2005, 15, 8366–8371.
72. Pan Q, Bao LW, Teknos TN, Merajver SD: Targeted disruption of protein kinase Cε reduces cell invasion and motility through inactivation of RhoA and RhoC GTPases in head and neck squamous cell carcinoma. Cancer Res, 2006, 66, 9379–9384.
73. Perletti GP, Folini M, Lin HC, Mischak H, Piccinini F, Tashjian AH Jr: Overexpression of protein kinase Cε is oncogenic in rat colonic epithelial cells. Oncogene, 1996, 12, 847–854.
74. Racke FK, Wang D, Zaidi Z, Kelley J, Visvader J, Soh JW, Goldfarb AN: A potential role for protein kinase C-ε in regulating megakaryocytic lineage commitment. J Biol Chem, 2001, 276, 522–528.
75. Ricart AD, Tolcher AW, Liu G, Holen K, Schwartz G, Albertini M, Weiss G et al.: Volociximab, a chimeric monoclonal antibody that specifically binds alpha5beta1 integrin: a phase I, pharmacokinetic, and biological correlative study. Clin Cancer Res, 2008, 14, 7924–7929.
76. Sakanoue Y, Hatada T, Kusunoki M, Yanagi H, Yamamura T, Utsunomiya J: Protein kinase C activity as marker for colorectal cancer. Int J Cancer, 1991, 30, 803–806.
77. Sampson SR, Cooper DR: Specific protein kinase C isoforms as transducers and modulators of insulin signaling. Mol Genet Metab, 2006, 89, 32–47.
78. Schultz A, Larsson C: Ceramide influences neurite outgrowth and neuroblastoma cell apoptosis regulated by novel protein kinase C isoforms. J Neurochem, 2004, 89, 1427–1435.
79. Shen GX: Selective protein kinase C inhibitors and their applications. Curr Drug Targets Cardiovasc Haematol Disord, 2003, 3, 301–307.
80. Slupsky JR, Kamiguti AS, Harris RJ, Cawley JC, Zuzel M: Central role of protein kinase Cε in constitutive activation of ERK 1/2 and Rac1 in the malignant cells of hairy cell leukemia. Am J Pathol, 2007, 170, 745–754.
81. Song MS, Park YK, Lee J, Park K: Induction of glucose-regulated protein 78 by chronic hypoxia in human gastric tumor cells through a protein kinase C-ε/ERK/AP-1 signaling cascade. Cancer Res, 2001, 61, 8322–8330.
82. Souroujon MC, Mochly-Rosen D: Peptide modulators of protein-protein interactions in intracellular signaling. Nat Biotechnol, 1998, 16, 919–924.
83. Stanwell C, Gescher A, Bradshaw TD, Pettit GR: The role of protein kinase C isoenzymes in the growth inhibition caused by bryostatin 1 in human A549 lung and MCF-7 breast carcinoma cells. Int J Cancer, 1994, 56, 585–592.
84. Stawowy P, Margeta C, Blaschke F, Lindschau C, Spencer-Hansch C, Leitges M, Biagini G et al.: Protein kinase C ε mediates angiotensin II-induced activation of β1-integrins in cardiac fibroblasts. Cardiovasc Res, 2005, 67, 50–59.
85. Steinberg SF: Structural basis of protein kinase isoform function. Physiol Rev, 2008, 88, 1341–1378.
86. Stensman H, Larsson C: Protein kinase Cepsilon is important for migration of neuroblastoma cells. BMC Cancer, 2008, 8, 365–378.
87. Sun MK, Alkon DL: Protein kinase C pharmacology: Perspectives on therapeutic potentials as antidementic and cognitive agents. Recent Pat CNS Drug Discov, 2006, 1, 147–156.
88. Teicher BA: Protein kinase C as a therapeutic target. Clin Cancer Res, 2006, 12, 5336–5345.
89. Traum O, Monia BP, Dean NM, Berk BC: PKC-ε is required for mechano-sensitive activation of ERK1/2 in endothelial cells. J Biol Chem, 1997, 272, 31251–31257.
90. Ueffing M, Lovric J, Philips A, Mischak H, Kolch W: Protein kinase C-ε associates with the Raf-1 kinase and induces the production of growth factors that stimulate Raf-1 activity. Oncogene, 1997, 15, 2921–2927.

91. Villalona-Calero MA, Ritch P, Figueroa JA, Otterson GA, Belt R, Dow E, George S et al.: A phase I/II study of LY900003, an antisense inhibitor of protein kinase C-α, in combination with cisplatin and gemcitabine in patients with advanced non-small cell lung cancer. Clin Cancer Res, 2004, 15, 6086–6093.
92. Wheeler DL, Reddig PJ, Ness KJ, Leith CP, Oberley TO, Verna AK: Overexpression of protein kinase Cε in the mouse epidermis leads to a spontaneous myoproliferative-like disease. Am J Pathol, 2005, 166, 117–126.
93. Wheeler DL, Li Y, Verna AK: Protein kinase Cε ultraviolet light-induced cutaneous damage and development of squamous cell carcinoma possibly through induction of specific cytokines in a paracrine mechanism. Photochem Photobiol, 2005, 81, 9–18.
94. Wu D, Foreman TL, Gregory CW, McJilton MA, Wescott GG, Ford OH, Alvey RF et al.: Protein kinase Cε has the potential to advance the recurrence of human prostate cancer. Cancer Res, 2002, 62, 2423–2429.
95. Wu D, Thakore CU, Wescott GG, McCubrey JA, Terrian DM: Integrin signaling links protein kinase Cε to the protein kinase B/Akt survival pathway in recurrent prostate cancer cells. Oncogene, 2004, 23, 8659–8672.
96. Xu TR, He G, Dobson K, England K, Rumsby M: Phosphorylation at Ser-729 specifies a Golgi localisation for protein kinase C epsilon (PKCε) in 3T3 fibroblasts. Cell Signal, 2007, 19, 1986–1999.
97. Xu TR, Rumsby MG: Phorbol ester-induced translocation of PKC epsilon to the nucleus in fibroblasts: identification of nuclear PKC epsilon-associating proteins. FEBS Letters, 2004, 570, 20–24.
98. Yonezawa T, Kurata R, Kimura M, Inoko H: PKC delta and epsilon in drug targeting and therapeutics. Recent Patents on DNA and Gene Sequences, 2009, 3, 96–101.
99. Zeidman R, Lofgren B, Pahlman S, Larsson C: PKCε, via its regulatory domain and independently of its catalytic domain, induces neurite-like processes in neuroblastoma cells. J Cell Biol, 1999, 145, 713–726.
100. Zeidman R, Troller U, Ragunath A, Pahlman S, Larsson C: Protein kinase Cε actin-binding site is important for neurite outgrowth during neuronal differentiation. Mol Biol Cell, 2002, 13, 12–24.

Received: March 19, 2010; **in the revised form:** May 24, 2010; **accepted:** June 10, 2010.