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Modulation of Protein Kinase C in Antitumor Treatment

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Contents

1 Introduction

Protein kinase C (PKC) is a family of serine/threonine specific protein kinases. The PKC isoenzymes can be classified into three groups: i) the conventional (cPKCs) α, βI, βII, and γ (require negatively charged phospholipids, diacylglycerol or phorbol ester, and calcium for optimal activation), ii) the novel (nPKCs) δ, ε, θ, η/L (mouse/human) and μ (require negatively charged phospholipids, diacylglycerol or phorbol ester, but no calcium), and iii) the atypical (aPKCs) λ/ι (mouse/human) and ζ (do not require calcium, diacylglycerol or phorbol ester, but only negatively charged phospholipids for optimal activity) (Nishizuka, 1995; Newton and Johnson, 1998). The PKC isoenzymes (Fig. 1) are characterized by four conserved (C1–C4) and five variable (V1–V5) domains (Stabel and Parker, 1991; Azzi et al., 1992; Hug and Sarre, 1993; Stabel, 1994). The regulatory domain consists of the C1 and the C2 region. C1 contains the pseudosubstrate region that can inhibit the enzyme by binding to the catalytic site (C4). In PKCμ, the pseudosubstrate domain is lacking. C1 also contains tandemly repeated cysteine-rich regions to which DAG (diacylglycerol), phorbol esters and bryostatins can bind. cPKCs and nPKCs contain two zinc fingers in the phorbol ester binding site, aPKCs are characterized by a single zinc finger. C2 contains the calcium binding region present only in cPKCs but not in nPKCs and aPKCs. Between the C2 and the C3 region the so called hinge region is situated which serves as cleavage site for calpain and trypsin during degradation. The C3 region is believed to be the ATP binding site and the C4 region the catalytic site.

PKC isoenzymes seem to play an important role in activation of signal transduction pathways leading to synaptic transmissions, the activation of ion fluxes, secretion, proliferation, cell cycle control, differentiation or tumorigenesis. PKC has become of major interest as target for therapeutic

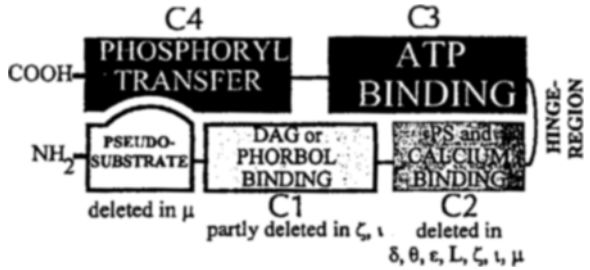

Fig. 1. Schematic representation of protein kinase C. The cartoon shows the different domains of PKC in the inactive form of the enzyme

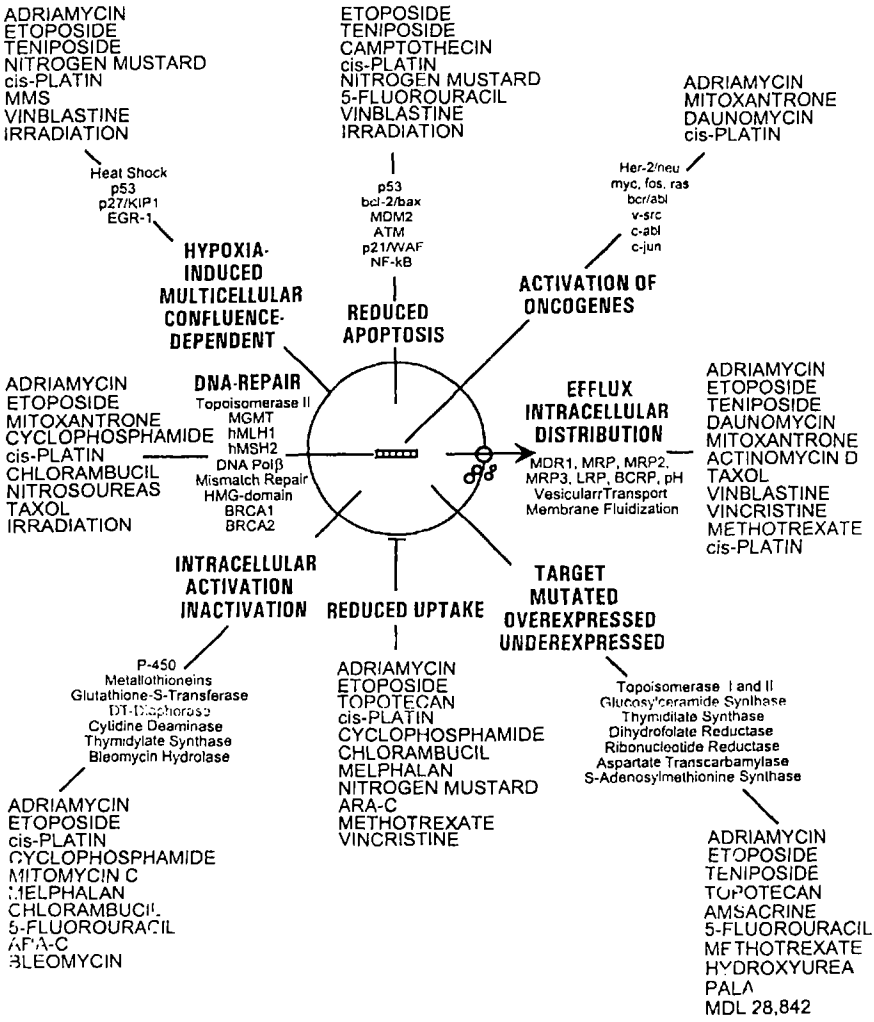

Fig. 2. Mechanisms causing resistance to antitumor treatment. ATM, ataxia telangiectasia gene, (Westphal et al., 1998; Xu and Baltimore, 1996), bcl-2/bax (Farrow and Brown, 1996, Zunino et al., 1997; Haq and Zanke, 1998), bcr/abl (McGahon et al., 1994), BCRP, breast cancer resistance protein (Doyle et al., 1998; Ross et al, 1999); bleomycin hydrolase (El-Deiry, 1997), BRCA1 (Husain et al., 1998; Chen et al., 1998), BRCA2 (Chen et al., 1998; Chen et al., 1999), c-abl (White and Prives, 1999), c-jun (Sanchez-Perez and Perona, 1999), cytidine deaminase (El-Deiry, 1997), DNA polβ, DNA polymerase β (Ochs et al., 1999), dihydrofolate reductase (Schimke, 1986), DT-diaphorase (Riley and Workman, 1992; Fitzsimmons et al., 1996; El-Deiry, 1997), EGR-1 (Ahmed et al., 1996), fos (Niimi et al., 1991), glucosylceramide synthase

intervention in a range of different diseases (Gescher et al., 1992; Bradshaw et al., 1993; Basu 1993; Deacon et al., 1997; Nixon, 1997; Goekjian and Jirousek, 1999). PKC may be involved in chronic granulomatous disease, allergy, asthma, rheumatoid arthritis (Westmacott et al, 1991), transplantation (Woodley et al., 1991), AIDS (Kinter et al., 1990; Accornero et al., 1998), Alzheimer's disease (Chauhan et al., 1991), multiple sclerosis (Defranco, 1991), hypertension (Ek et al., 1989), cardiac hypertrophy (Kwiatkowska-Patzer and Domanska-Janik, 1991), atherosclerosis (Kariya et al., 1987), diabetes (Inoguchi et al., 1992; Ishii et al., 1996) and cancer (Basu, 1993; Blobe et al., 1994, Gescher, 1998). PKC is the intracellular receptor for tumor promoting phorbol esters (Castagna et al., 1982; Niedel et al., 1983; Leach et al., 1983). Short term exposure of intact cells with phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (phorbol-12-myristate-13-acetate, TPA) activates PKC, long term exposure down-regulates PKC activity. Phorbol esters are able to promote tumor formation. Therefore, it was presumed that activation of PKC by TPA induces tumors and inhibition may reduce carcinogenesis or inhibit tumor growth. However, investigations revealed that the situation is more complicated. For example, bryostatin 1, another PKC

(Lavie et al., 1997; Liu et al., 1999), glutathione-S-transferase (Ozols et al., 1990, Tew,1994), heat shock (Ciocca et al., 1993); her-2/neu (Tsai et al., 1996), HMG-domains (Huang et al., 1994), hMLH1 (De las Alas et al. 1997), hMSH2 (Aebi et al., 1996), hypoxia (Bush et al., 1978; Vaupel et al., 1989; Sakata et al., 1991; Höckel et al., 1996), LRP, lung resistance-related protein (Scheffer et al., 1995), MDL 28,842, (*Z*)-5′-fluoro-4′,5′-didehydro-5′-deoxyadenosine (Dwivedi et al., 1999), mdm2 (Kondo et al., 1995a), MDR1 (Juliano and Ling, 1976; Gottesman and Pastan, 1993), membrane fluidization (Regev et al., 1999), metallothioneins (Kelley et al., 1988; Kaina et al., 1990; Kondo et al., 1995b), MGMT, O^6-methylguanine-DNA methyltransferase (Erickson, 1991; Mattern et al., 1998), methyl methanesulfonate (MMS; Chen et al., 1998), mismatch repair (Moreland et al., 1999, White and Prives, 1999), MRP, multidrug resistance-associated protein (Cole et al., 1994), MRP2 (Cui et al., 1999; Hooijberg et al., 1999), MRP3 (Kool et al., 1999), multicellular resistance (Sutherland, 1988; Kobayashi et al., 1993; Pizao et al., 1993; Graham et al., 1994; StCroix et al., 1996), myc (Sklar and Prochownik, 1991), NF-κB (Wang et al., 1999b), P-450 (Doehmer et al., 1993), p53 (Lowe et al., 1993; Levine, 1997; Zunino et al., 1997; Piovesan et al., 1998); p27/KIP (StCroix et al., 1996); p21/WAF1 (Wang and Walsh, 1996; McDonald et al., 1996; Fan et al., 1997), pH (Martinez-Zaguilan et al, 1999; Williams et al., 1999), ras (Scanlon et al., 1991; Isonishi et al., 1991; El-Deiry, 1997), reduced uptake (Fry and Jackson, 1986; Perez et al., 1990; Slapak et al., 1990; Chu, 1994; Ma et al., 1998a; Moscow, 1998; Ma et al., 1998b), repair (Masumoto et al., 1999; Chen et al., 1998; Husain et al., 1998, Chen et al., 1999), ribonucleotide reductase (Ask et al, 1993; Yen et al., 1994), thymidilate synthase (Kinsella et al., 1997), topoisomerase I and II (Yarbro, 1992; Robert and Larsen, 1998), vesicular transport (Dietel et al., 1990), v-src (Masumoto et al., 1999)

modulator with properties similar to those of TPA (Blumberg, 1991; Kennedy et al., 1992; Szallasi et al., 1994) does not induce tumor formation. The compound exhibits potent antitumor activity and is currently undergoing phase I (Jayson et al., 1995, Grant et al., 1998) and phase II (Propper et al., 1998) clinical evaluation as an anticancer drug.

In addition to be a direct target of antitumor treatment, PKC has been shown to be involved in the resistance to antitumor treatment and in the modulation of apoptosis. Resistance to cancer chemotherapy is a major

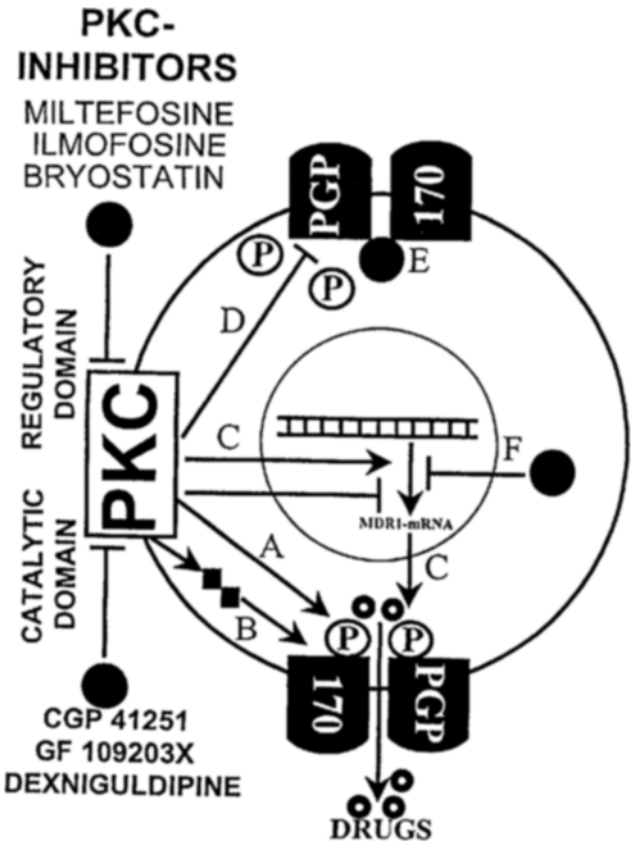

Fig. 3. Possible interactions between PKC and MDR1-mediated drug resistance. Activation of PKC might activate the drug efflux by phosphorylation of PGP (A), induce or activate proteins which modulate PGP (B, Castro et al., 1999), or induce the transcription and translation of MDR1-mRNA (C). Inhibitors of PKC might prevent phosphorylation of PGP leading to a decrease the drug efflux (D), inhibit the efflux of drugs by direct interaction with the drug binding site(s) or the ATP-binding sites of PGP (E), or prevent the expression of MDR1-mRNA (F)

problem in the treatment of cancer (Goldie and Coldman, 1984). A variety of mechanisms causing resistance have been observed (Fig. 2), among them: enhanced DNA-repair, reduced drug uptake, intracellular inactivation of drugs, reduced prodrug activation, mutated, overexpressed or not expressed targets, hypoxia, cell-cell interactions (multicellular resistance), increased drug efflux by overexpression of multidrug resistance gene 1 (MDR1), multidrug resistance-associated gene (MRP) or lung resistance protein (LRP), export of drugs by vesicles, intracellular compartimentalization of drugs, and altered expression or mutation of genes involved in apoptosis. Members of the PKC family have been reported be involved in multidrug resistance (MDR) and in apoptosis. Figure 3 shows an overview of the possible mechanisms by which PKC might interfere with MDR1-mediated drug resistance. However, the results of investigations concerning PKC and resistance are contradicting. This review summarizes data indicating a role of PKC in antitumor treatment and apoptosis, and on the other hand, data that argue against an involvement of PKC. The association of experimental results with pro or contra of a contribution of PKC is not always unambiguous.

2 PKC in Cell Proliferation and Tumor Growth

2.1 PKC Isoenzymes and Cell Proliferation

Investigations into the expression of distinct PKC isoenzymes in various tissues revealed a highly variable tissue distribution. PKCα and PKCζ are ubiquitously expressed. Brain contains all isoenzymes, whereas others such as skin and skeletal muscle contain only a few (Blobe et al., 1994). Such a different pattern of expression suggests that the PKC isoenzymes play different roles in the tissue of expression and do not suggest a general role of all isoenzymes in cell proliferation. In many publications an influence of PKC on cell proliferation has been reported.

Deletion or mutation of PKC1, the only member of the PKC family expressed in Saccharomyces cerevisiae (Mellor and Parker, 1998) led to osmotic instability, an arrest of protein synthesis and cell proliferation (Levin et al., 1990; Levin and Bartrett-Heubusch, 1992). NIH3T3 cells overexpressing PKCα exhibited an altered, "transformed-like" morphology, an increased growth rate, a higher saturation density and were able to grow in soft agar after treatment with TPA. These effects could be reversed by the unspecific PKC inhibitor staurosporine (Finkenzeller et al., 1992). With respect to untreated control liver, an activation and increased expression of PKCα was observed in diethylnitrosamine-induced liver tumors and lung metastases (La Porta et al., 1997). Early events in the transformation of keratinocytes

have been found to be the mutation and activation of ras, activation of the epidermal growth factor receptor, upregulation of PKCα, inactivation of through tyrosine phosphorylation (Yuspa, 1998), and downregulation of the expression of PKCδ (Geiges et al., 1995). In cultured myoblasts PKCα was found to have an important role in maintaining proliferation (Capiati et al., 1999). PKCα has been shown to activate telomerase in human breast cancer cells which may represent an essential step in the maintenance of proliferation in human cancers (Li et al., 1998). On the other hand, increased expression of PKCα led to cessation of growth, induction of differentiation in B16 melanoma cells and to gene dose-dependent inhibition of proliferation in K562 cells (Gruber et al., 1992). Recombinant chimaeras with the regulatory domain of PKCα and catalytic domains of other PKC isoenzymes inhibited cell growth (Acs et al., 1997). PKCα overexpressing bovine aortic endothelial cells exhibited reduced proliferation and increased accumulation in the G2/M phase of the cell cycle (Rosales et al., 1998). Overexpression of PKCα in MCF10A cells suppressed proliferation endowing cells with properties consistent with a metastatic phenotype (Sun and Rotenberg, 1999).

PKCβ overexpressing cells were more susceptible to transformation with the H-ras oncogene (Hsiao et al., 1989). Overexpression of PKCβI in rat fibroblasts led to disordered growth (Housey et al., 1988). The human erythroleukemia K562 cell line expresses PKCα, βII and ζ. The cells undergo megakaryocytic differentiation and cessation of proliferation when treated with TPA. K562 cells overexpressing human PKCα grew more slowly and were more sensitive to the cytostatic effects of TPA than control cells, whereas cells overexpressing βII were less sensitive to TPA. Antisense experiments demonstrated that PKCβII is required for K562 cell proliferation, whereas PKCα is involved in megakaryocytic differentiation (Murray et al., 1993). In F9 embryonal carcinoma PKCα seems to play an active role in differentiation and PKCβ activity is incompatible with differentiation (Cho et al., 1998). PKCβI overexpressing bovine aortic endothelial cells promoted growth and shortened the doubling time, whereas PKCα exhibited reduced proliferation (Rosales et al., 1998). However, in human HL-60 promyelocytes activation of PKCβ was found to be necessary and sufficient for TPA-induced differentiation (MacFarlane and Manzel, 1994). In murine keratinocytes overexpression of βI led to growth inhibition and Ca^{2+}-induced differentiation (O'Driscoll et al., 1994). PKCβI expression was higher in a well-differentiated SKUT-1-B mixed mesodermal uterine cell line compared to the moderately differentiated endometrial HEC-1-B adenocarcinoma cell line (Bamberger et al., 1996). As shown in mice homozygous for a targeted disruption of the PKCβII gene, this isoenzyme seems to play an important role in B-cell activation (Leitges et al., 1996).

When PKCγ was overexpressed in NIH 3T3 cells, reduced growth factor requirements, growth to higher saturation density and formation of tumors in nude mice were observed (Persons et al., 1988). Human U251 MG glioma cells overexpressing PKCγ showed an increased rate of growth in monolayer culture, increased colony-forming efficiency on soft agar, and increased DNA synthesis in response to epidermal growth factor and basic fibroblast growth factor (Mishima et al., 1994).

PKCδ seems to be involved in growth inhibition, differentiation, apoptosis and tumor suppression (Gschwendt, 1999). TPA stimulation of PKCδ overexpressing CHO cells led to a cell division arrest (Watanabe et al., 1992). PKC δ overexpressing glioma cells showed a decreased rate of growth and decreased colony-forming efficiency (Mishima et al, 1994). However, overexpression of PKCδ in rat HT mammary adenocarcinoma cell lines significantly increased anchorage-independent growth, although it had no effect on growth of adherent cells. PKCδ seems to be involved in regulating attachment and anchorage-independence, which may be related to increased metastatic potential in this system (Kiley et al., 1999). Recombinant chimaeras with the regulatory domain of PKCδ and catalytic domains of other PKC isoenzymes inhibited cell growth (Acs et al., 1997). Proteolytic cleavage of PKCδ was found to activate this isoenzyme during apoptosis (Emoto et al., 1995; Ghayur et al., 1996). The antitumor agents taxol, vinblastine and vincristine specifically activated PKCδ (Das et al., 1998). Transgenic mice overexpressing PKCδ in the epidermis were found to be resistant to skin tumor promotion by TPA (Reddig et al., 1999). In NIH 3T3 cells which normally express only PKCα, overexpression of PKCδ by transfection induced significant changes in morphology and caused the cells to grow more slowly and to a decreased cell density in confluent cultures. These changes were accentuated by treatment with TPA. (Mischak et al., 1993). Overexpression of PKCε did not lead to morphological changes, but caused increased growth rates and higher cell densities in monolayers. None of the PKCδ overexpressers grew in soft agar with or without TPA, but all the cell lines that overexpressed PKCε grew in soft agar in the absence of TPA, but not in its presence. NIH 3T3 cells that overexpressed PKCε also formed tumors in nude mice with 100% incidence, indicating that high expression of PKCε contributes to neoplastic transformation (Mischak et al., 1993). Overexpression of PKCε in Rat 6 embryo fibroblasts led to a 7–13-fold increase in Ca^{2+}-independent PKC activity, to formation of dense foci in monolayer culture, decreased doubling time, increased saturation density, decreased serum requirement, growth in soft agar, and tumor formation in nude mice (Cacace et al., 1993). In nontumorigenic rat colonic epithelial cells overexpression of PKCε caused marked morphological changes in two transfected

clones, which were accompanied by increased saturation densities and anchorage-independent colony formation in semisolid agar. These growth effects were attenuated or reversed by chronic incubation with TPA (Perletti et al., 1996). A small cell lung cancer cell line that exhibited rapid growth compared to other small cell lung cancer cell lines overexpressed a constitutively active catalytic fragment of PKCε (Baxter et al., 1992). Dominant negative PKCε inhibited the proliferation of NIH3T3 cells. Constitutively active PKCα or PKCε overcame this inhibitory effect (Cai et al., 1997). Recombinant chimaeras between the regulatory domains of PKCα, PKCδ, PKCε and the catalytic domains of these isoenzymes were transfected into NIH 3T3 cells. All chimaeras containing a regulatory or a catalytic domain of PKCε exhibited growth-promoting activity (Acs et al., 1997). These data indicate that PKCε seems to have oncogenic properties. However, it has also been reported, that inhibition of cell proliferation by tamoxifen is associated with activation of PKCε (Lavie et al., 1998). In neuronal cells PKCε seems to be important for differentiation (Ohmichi et al., 1993; Hundle et al., 1995; Fagerstrom et al., 1996).

Overexpression of PKCζ has been reported to be required for mitogenic maturation of Xenopus oocytes and led to deregulation of growth control in mouse fibroblasts (Berra et al., 1993). However, these effects of PKCζ in Xenopus oocytes seem not to be clear (Carnero et al., 1995). In U937 monocytic leukemia cells PKCζ overexpression decreased proliferation rate and saturation density, indicating the induction of differentiation (Ways et al., 1994). In normal NIH3T3 fibroblasts (Montaner et al., 1995; Crespo et al., 1995) and K562 cells (Murray et al., 1997) no effects of PKCζ overexpression on cell proliferation or oncogenic transformation were observed. PKCζ overexpression in v-raf-transformed NIH-3T3 cells drastically retarded proliferation, abolished anchorage-independent growth, and reverted the morphological transformation (Kieser et al., 1996). Activation of PKCζ by ceramide in acute lymphoblastic leukemia MOLT-4 cells induced apoptosis. However, it was also shown that ceramide treatment, in addition to activation of PKCζ, inactivated PKCα (Lee et al, 1996a). Exposure of cells to a genotoxic stimulus that induced apoptosis, led to an inhibition of PKCζ (Berra et al, 1997). The product of the par-4 gene interacted with PKCζ and inhibited its enzymatic activity. The expression of par-4 correlated with growth inhibition and apoptosis (Diaz-Meco et al, 1996). In Cos-7 cells conventional and novel PKCs activated the ERK/MAPK cascade via raf-1, whereas PKCζ stimulated this pathway without raf-1 activation (Schönwasser et al., 1998). N-myc acts to increase the malignancy of neuroblastoma cells. Overexpression of N-myc in these cells caused suppression of PKCδ and induction of PKCζ (Bernards, 1991).

PKCμ seems to be involved in murine keratinocyte proliferation. A correlation between PKCμ expression and enhanced cell proliferation was also observed for NIH3T3 mouse fibroblasts overexpressing human PKCμ (Rennecke et al., 1999).

2.2 PKC Expression in Tumor Cells and Tumors

Overexpression of PKC seems to be involved in breast cancers. Elevated levels of PKC activity in breast tumors relative to normal breast tissue was found by O'Brian et al. (1989a). TPA inhibited the growth of mammary carcinoma MCF-7, BT-20, MDA-MB-231, ZR-75-1, and HBL-100, but not that of T-47-D cells. TPA-non-responsive T-47-D cells exhibited the lowest PKC activity. A rapid TPA-dependent translocation of cytosolic PKC to membranes was found in the five TPA-sensitive cell lines without affecting cell growth. However, TPA-treatment for more than 10 hours inhibited reversibly the growth of TPA-responsive cells. This effect coincided with the complete loss of cellular PKC activity due to the proteolysis of the translocated membrane-bound PKC. Resumption of cell growth after TPA-removal was closely related to the specific reappearance of the PKC activity in the TPA-responsive human mammary tumor cell lines suggesting an involvement of PKC in growth regulation (Fabbro et al., 1986). During a 4-day culture period, various phorbol ester derivatives inhibited the proliferation of MCF-7 breast carcinoma cells in a dose-dependent manner. A correlation between the relative potencies of the various phorbol ester derivatives for inhibiting both phorbol-12,13-dibutyrate (PdBu) binding and cell proliferation was found (Darbon et al., 1986). However, it was also reported that PKC activation by TPA and DAG inhibited MCF-7 cell proliferation (Issandou and Darbon, 1988; Issandou et al., 1988). PKC activity was described to be higher in estrogen receptor negative human mammary tumor cells compared to estrogen-receptor-containing counterparts (Borner et al., 1987; Ways et al., 1995; Morse-Gaudio et al., 1998). MCF-7 cells transfected with PKCα displayed an enhanced proliferative rate, anchorage-independent growth, dramatic morphologic alterations including loss of an epithelioid appearance, and increased tumorigenicity in nude mice. PKCα overexpressing MCF-7 cells exhibited a significant reduction in estrogen receptor expression and decreases in estrogen-dependent gene expression (Ways et al., 1995). Phorbol esters were found to down-regulate the expression of estrogen receptors in breast cancer cell lines (Hähnel and Gschwendt, 1995). PKCα was found to be activated in situ in a significant number of human breast tumors (Ng et al., 1999). MCF-7 breast cancer cells transfected with PKCα led to a more aggressive phenotype compared to untransfected cells (Ways et al., 1995).

Differential display between the non-metastatic and the PKCα overexpressing metastatic MCF-7 cells showed that a homologue to a putative glialblastoma cell differentiation-related protein is upregulated. Histone 3B, integrins 3α and 6α were downregulated (Carey and Noti, 1999). In contrast to the publication by Ways et al. (1995), another report showed that overexpression of PKCα in MCF-7 cells caused upregulation of PKCβ and this led to a less aggressive phenotype, which was characterized by reduced in vitro invasiveness and markedly diminished tumor formation and growth in nude mice. These findings were explained by the down-regulation of estrogen receptor levels observed in tumors derived from PKCα-infected MCF-7 cells (Manni et al., 1996). TPA and bryostatin 1 inhibited the growth of MCF-7 breast cancer cells. TPA induced rapid translocation of PKCα protein and PKC activity to the membrane fraction of MCF-7 cells. In contrast, bryostatin 1 treatment resulted in the loss of the PKCα activity from both cytosolic and membrane compartments within 10 minutes of treatment. These results suggested that PKCα may specifically play a role in inhibiting growth of human breast cancer cells by bryostatin (Kennedy et al., 1992).

PKCα expression in human astrocytomas was found to be highest in well-differentiated (grade 1) tumors, intermediate in anaplastic (grade 2) astrocytomas, and low or nondetectable in dedifferentiated glioblastomas (grade 3 astrocytomas) and normal controls (Benzil et al., 1992). It was also found that the levels of PKCα in eight glioblastoma cells lines were similar to those in normal glial cells (Misra-Press et al., 1992). PKC activity was significantly higher in glioma cell lines compared to bladder, colorectal, rhabdomyosarcoma-oligodendrocyte hybrid, melanoma, cervix, and fibroblast cells, even though 3 of 8 of the non-glioma lines had higher proliferation rates than A172 glioma cells. In non-glioma cell lines, no correlation was found between the PKC activity and proliferation rates (Baltuch et al., 1993). In a comparison between rat C6 glioma cells and non-malignant rat astrocytes, both C6 glioma cells and astrocytes were found to express PKCα, β, δ, ε and ζ, but not γ. Enzyme activity measurements revealed that the elevated PKC activity of glioma cells was due to overexpression of PKCα (Baltuch et al., 1995). In another investigation all human glioma lines examined, and the rat glioma C6, displayed high PKC activity relative to nonmalignant glial cells, which correlated with their proliferation rates over their respective growth phase. Frozen surgical human malignant glioma specimens also displayed high PKC activity (Couldwell et al., 1992). The administration of PdBu or TPA resulted in a dose-related inhibition of growth of human glioma cell lines in vitro. The synthetic nonphorbol PKC activator SC-9 produced an even more pronounced decrease in cell proliferation. Conversely, the administration of 4-αTPA, a phorbol ester that binds but does not activate

PKC, had no effect on the proliferation rate. In contrast to the response of glioma cells, nonmalignant human adult astrocytes treated with the PKC activators responded by increasing their proliferation rate. The opposed effects of PKC activators on nonmalignant astrocytes versus glioma growth may be due to a high intrinsic PKC activity in glioma cells, with resultant down-regulation of enzyme activity following the administration of the pharmacological activators (Couldwell et al., 1990). Cell lines derived from high-grade gliomas expressed higher levels of PKCα than did cell lines derived from low-grade gliomas. In glioblastoma-derived cell lines PKCα was mainly expressed in the cytosolic fraction, indicating an inactive state of the enzyme. Bryostatin 1 specifically down-regulated PKCα in glioblastoma-derived cell lines. However, this was not associated with significant growth inhibition illustrating that PKCα seems not to be essential for proliferation (Zellner et al., 1998). Compared to two normal glial cell lines, in eight glioblastoma cell lines PKCα and PKCγ were similar. PKCε was elevated three to thirty times in six of the eight tumors. PKCζ was elevated twofold in all of the tumors (Xiao et al., 1994).

Elevated levels of PKC were also found in thyroid cancers if compared to normal thyroid tissue (Hagiwara et al., 1990; Hatada et al., 1992). TPA enhanced, tamoxifen and staurosporine inhibited invasion and growth of estrogen receptor-negative follicular thyroid cancer cells (Hoelting et al., 1996). In human thyroid cancers (Prevostel et al., 1995; Prevostel et al., 1997) and in pituitary cancers (Alvaro et al., 1997; Alvaro et al., 1993) point mutations in PKCα were detected. However, in 11 human pituitary tumours cDNA was subcloned and up to ten individual clones were sequenced from each tumour, resulting in 85 clones analyzed in total. All of the pituitary adenomas showed a normal wild-type sequence of PKCα DNA. Even if the tumor was invasive (infiltration of the dura mater) no mutation was found. Moreover, Western blot analyses did not show any differences in PKCα protein expression in invasive as compared with noninvasive pituitary adenomas. These data argue against suggestions that mutated PKCα is a feature of invasive pituitary tumours (Schiemann et al., 1997). PKC activity and expression were higher in adenomatous pituitaries than in normal human or rat pituitaries. PKC expression in growth hormone-secreting and non-secreting tumors was significantly higher than that in prolactin-secreting tumors. PKC activity was significantly higher in invasive tumors than in non-invasive tumors. In 3 adenomas which were obtained from patients treated with bromocriptine or octreotide, particulate- and soluble-PKC activities were significantly lower than those measured in non-treated adenomas (Alvaro et al., 1992). In human pituitary tumors predominantly PKCα in all adenomas, and variable expression of PKCβ and PKCγ in some tumors

was found. Normal and neoplastic pituitaries expressed abundant mRNA for PKCε, whereas some tumors and one normal pituitary had a few cells positive for PKCζ (Jin et al., 1993).

A murine UV-induced fibrosarcoma cell line had an unusual PKC subcellular distribution with 87% of the PKC activity associated with the membrane. Sequencing of PKCα DNA from ultraviolet-induced-fibrosarcoma cells showed four point mutations in the fibrosarcoma PKC, of which three are in the highly conserved regulatory domain and one is in the conserved region of the catalytic domain. Expression of this mutant PKCα gene in normal Balb/c 3T3 fibroblasts resulted in a fibrosarcoma-like PKC membrane localization and in cell transformation, as judged by their formation of dense foci, anchorage-independent growth and ability to induce solid tumours when inoculated into nude mice. By contrast, transfectants expressing the normal PKCα cDNA did not display a morphology typical of malignant transformed cells and failed to induce tumours in vivo. These findings seemed to demonstrate that point mutations in the primary structure of PKC modulate enzyme function and are responsible for inducing oncogenicity (Megidish and Mazurek, 1989). However, these results could not be reproduced (Borner et al., 1991). The nontumorigenic, immortal line of murine melanocytes, Mel-ab, required the continual presence of biologically active phorbol esters for growth. Comparable treatments of murine B16 melanoma cells resulted in partial inhibition of cell proliferation. Significant levels of PKC were present in quiescent Mel-ab cells, whereas no immunoreactive protein was detected in cell extracts from either proliferating Mel-ab or B16 cells. These data showed that PKC down-regulation, and not activation, correlates with the growth of melanocytes in culture (Wilson et al., 1989; Brooks et al., 1991). It has been shown that TPA stimulated the proliferation of normal human melanocytes, whereas it inhibited the growth of human melanoma cell lines. PKCδ, ε and ζ were detected in both normal melanocytes and in four melanoma cell lines. In contrast, both PKCα and β were expressed in normal melanocytes, whereas only either PKCα or β was detected in melanoma cells. TPA inhibited the growth of cells lacking PKCα more efficiently than the other melanoma cell lines which lacked PKCβ. It was further shown that PKCβ was not detected in freshly isolated human primary or metastatic melanoma tissues. (Oka et al., 1996). This may be a consequence of lack of induction of terminal differentiation by PKCβ. In human early prostatic adenocarcinomas an increase of PKCα, PKCε, PKCζ and a decrease of PKCβ was consistently observed during the genesis and progression of prostate cancer compared with nonneoplastic prostate tissues (Cornford et al., 1999). An increased nuclear PKCβ activity was observed in lung metastases compared to the parental liver tumor induced by diethylni-

trosamine (La Porta et al., 1997). Spontaneously and chemically transformed mouse pulmonary epithelial cells exhibited reduced levels of PKC (Morris and Smith, 1992).

Underexpression of PKC seems to be involved in colon cancer. PKC has been shown to be important for growth arrest and differentiation of intestinal cells (Saxon et al., 1994, Assert et al., 1993). Human Vaco 10 MS colon cancer cells were growth-inhibited by activation of PKC (McBain et al., 1990). Significantly higher Ca^{2+}-dependent PKC activities were observed in both the cytosolic and particulate fractions of the normal mucosa relative to the corresponding values obtained with the human colon carcinoma fractions. The average specific activity ratios were 5.1 (normal cytosolic/carcinoma cytosolic) and 3.7 (normal particulate/carcinoma particulate) for PKC (Guillem et al., 1987). In patients with colonic adenomas and colonic carcinomas, total PKC activity was found to be significantly reduced as compared to adjacent mucosa (Kopp et al., 1991). In 15 of 15 primary human colon tumors there was a decrease of approximately 40% in the levels of diacylglycerol when compared to paired adjacent normal mucosa samples. Assays on the same samples indicated that this decrease was seen both in tumors that did and did not display mutations in codon 12 of c-K-ras. These results suggested that the PKC signal transduction pathway is suppressed in human colon cancer (Phan et al., 1991). In another investigation the mean value for cellular PKC enzyme activity in the colon tumors from 39 patients was approximately 60 percent of that found in the paired adjacent normal mucosa samples (Levy et al., 1993). In 18 human colonic adenomas and carcinomas a significant decrease in particulate PKC activity compared with the adjacent normal mucosa was observed. Decreased PKC activity correlated with increased adenoma size (Kusunoki et al., 1992). Five of six PKC isoenzymes present in normal mucosa showed reduced protein levels during tumor development in the human colon (Kahl-Rainer et al., 1994). Decrease of PKCα seems to be of major importance for development of colorectal cancers (Suga et al., 1998). DNAs for PKCα in sense or antisense orientations were transfected into human colonic adenocarcinoma CaCo-2 cells. Sense transfected clones exhibited 3-fold increases and antisense transfectants approximately 95% decreases in PKCα expression with no significant alterations in other PKC isoforms. Transfection of CaCo-2 cells with PKCα in the antisense orientation resulted in enhanced proliferation and decreased differentiation, as well as in a more aggressive transformed phenotype compared with empty vector-transfected control cells. In contrast, cells transfected with PKCα cDNA in the sense orientation demonstrated decreased proliferation, enhanced differentiation, and an attenuated tumor phenotype compared with these control cells. (Scaglione-Sewell et al., 1998). Human