ELSEVIER

European Journal of Cancer 40 (2004) 10–20

European Journal of Cancer

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Review

The role of protein kinase C-alpha (PKC-α) in malignancies of the gastrointestinal tract

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Received 19 June 2003; received in revised form 19 July 2003; accepted 15 August 2003

Abstract

Drugs specifically designed to block cellular signalling proteins are currently evaluated as a new way to treat gastrointestinal tumours. One such "new targeted agent" is aprinocarsen, an antisense oligonucleotide that specifically blocks the mRNA of protein kinase C-alpha (PKC-α). Blocking PKC-α, an important cellular signalling molecule associated with tumour growth, is anticipated to result in tumour cell arrest and achieve clinical benefits. However, it is not known which patients may benefit most from a specific inhibition of PKC-α. Past experience with other novel targeted agents suggests that expression of the target molecule is an important factor for the success of such a specific therapy. Therefore, reviewing the specific role of PKC-α in various gastrointestinal tumours may contribute to focus the clinical development of selective or specific PKC-α inhibitors, such as aprinocarsen, on those patients with a distinctive PKC-α expression pattern.

1. Introduction

Recent advances in cancer biology have led to the development of novel drugs in oncology, so called 'new targeted agents'. A recent example of these specific or targeted agents is imatinib mesylate for the treatment of gastrointestinal stromal tumours (GIST) [1]. Similar to imatinib mesylate, targeted agents have been developed to block signalling proteins, such as protein kinase C-alpha (PKC-α). While various reports support, in general, the role of PKC-α in cancer [2], its specific role in various gastrointestinal tumour types is not well recognised. Because selective PKC-α inhibitors and the specific PKC-α inhibitor, aprinocarsen (AFFINITAK™, LY900003, ISIS 3521) have been developed [3], a good understanding of the distribution and degree of PKC-α expression in gastrointestinal tumours is important for the clinical development of such targeted agents [4]. Therefore, this review on the role of PKC-α in gastrointestinal tumours may contribute to identify those patients that will most likely benefit from a specific PKC-α inhibitor, such as aprinocarsen.

2. General overview of PKC-α and the protein kinase C family of signalling proteins

Protein kinase C (PKC) is a phospholipid-dependent, cytoplasmic, serine/threonine kinase that is involved in intracellular signal transduction [2,5,6]. PKC is a family of isoenzymes that are being phosphorylated, or activated, in response to growth factors, hormones and neurotransmitters [2,5,7]. PKC isozymes have been classified into three groups: Group A or classical (PKC-α, βI, βII, and γ) that require a lipid cofactor (e.g. phosphatidyl-serine, PS), calcium (Ca^{2+}) and 1,2-diacylglycerol (DAG) for activation; Group B or new (PKC-δ, ε, η, θ, and μ) that are Ca^{2+}-independent; and Group C or atypical (PKC-λ and ζ) that require only PS [6].

The α-isozyme of PKC (PKC-α) has been located on chromosome 17q22-23.2 [8] and its protein is widely expressed in various tissues. Abnormal levels of PKC-α have been found in many transformed cell lines and in several human tumours [2,9–16]. After phosphorylation,

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doi:10.1016/j.ejca.2003.08.020

PKC-α translocates to cytosolic or nuclear membranes, leading to activation of a myriad of proteins that trigger such cellular responses as proliferation, differentiation, membrane transport, and gene expression [7]. Pharmacological inhibitors of PKC-α decrease growth and survival of tumours, reduce neoplastic properties, promote apoptosis, and sensitise tumour cells to chemotherapeutic agents [2,17,18]. The present review will examine which of the above-mentioned properties of PKC-α play a specific role in the tumorigenesis of gastrointestinal cancer [19–23].

2.1. The Role of PKC-α in tumours of the gastrointestinal tract (GI)

Worldwide gastrointestinal tumours are one of the most common tumours. Depending on their anatomical location, incidences of gastrointestinal tumours vary by geographical region (Table 1) [24,25]. While colorectal tumours are still frequent in the United States of America (USA), gastric and hepatic cancers represent a worldwide health challenge. We review here the role of PKC-α for each of the gastrointestinal tumours to better characterise which tumour type most commonly expresses PKC-α.

2.2. Oesophageal cancer

The incidence of oesophageal cancer has been increasing since the 1980s, mainly due to an increase in the number of oesophageal adenocarcinomas. Risk factors include high alcohol and cigarette consumption, exposure to nitrosamines, achalasia, obesity and reflux oesophagitis [26].

Table 1
Indicence of gastrointestinal tumours in the US and World

GI cancer	Cases per year ACS 2003 estimates WHO 2000 totals	Deaths per year ACS 2003 estimates WHO 2000 totals
Oesophageal cancer	13 900 412 327	13 000 337 501
Gastric cancer	22 400 876 341	12 100 646 567
Pancreatic cancer	30 700 216 367	30 000 213 462
Liver cancer	17 300 564 336	14 400 548 554
Gall bladder cancer	6800 N/A	3500 N/A
Colon/rectal cancer	105 500 944 717	N/A 491 411

GI, gastrointestinal; WHO, World Heath Organization; N/A, not available; US, United States; ACS, American Cancer Society.

Genetic imbalances in oesophagus carcinoma are common and complex with frequent loss of chromosomes 17p and 5q [27]. The loss of 17p is associated with poor survival, possibly due to the loss of wild-type p53 [28]. During progression of oesophageal tumours, chromosome 17q gains are sometimes observed [29]. This suggests an amplification of genes located on 17q and may explain why HER-2/neu has been associated with poor survival in Barrett's-syndrome-associated adenocarcinoma [30]. Some studies have investigated the role of PKC-α on the tumorigenesis of oesophageal cancer [31]. However, these studies are few and some even suggest that PKC-ε and not PKC-α plays a role in oesophagus cancer [32]. Clearly, further studies are needed to understand the role of PKC-α in oesophageal cancer.

2.3. Gastric cancer

Although the incidence of gastric cancer has decreased over the past 60 years, the prognosis remains poor, in part because gastric cancer is often chemoresistant. Predisposing factors include nitrate-rich food, cigarette consumption and *Helicobacter pylori* infection. Over 95% of all stomach cancers are adenocarcinoma [33]. Genetic imbalances in gastric cancer are complex and usually show gains of chromosomes including chromosome 17 [34–36]. Thus, it is intriguing to speculate whether gains of chromosome 17 contribute to increased PKC-α levels leading to multi-drug resistance (MDR). In fact, several *in vitro* studies suggest that PKC-α overexpression contributes to MDR, apoptosis and tumour cell proliferation [37] (Fig. 1).

For instance, using the SGC7901 gastric cancer cell line, one study examined the relationship of MDR and PKC-α expression after hypotonic exposure [38]. Compared with the SGC7901 parental cell line, the multidrug-resistant cell line, SGC7901/VCR, showed a threefold increased expression of PKC-α, while expression of other PKC isoforms were unchanged (PKC-β_I, PKC-β_{II} and PKC-γ) [39]. Additional studies in these cell lines showed that vincristine increased the expression of PKC-α prior to the development of MDR [40]. A similar observation was seen in the MKN-74 gastric cell line. In these cells, mitomycin induced expression of PKC-α and anti-apoptotic proteins, such as cyclo-oxygenase-2 (COX-2) and Bcl_2. The authors of this study concluded that MDR was due to the enhancement of the anti-apoptotic pathway and not to a direct stimulation of the *mdr* gene or its related genes [41]. In contrast to these observations on PKC-α-mediated anti-apoptosis, PKC-α can also induce apoptosis. This induction of apoptosis was seen in gastric cancer cell lines, MKN45 and MKN74, after they had lost anchorage dependence. The PKC-α-dependent apoptosis was attributed to an increased expression of

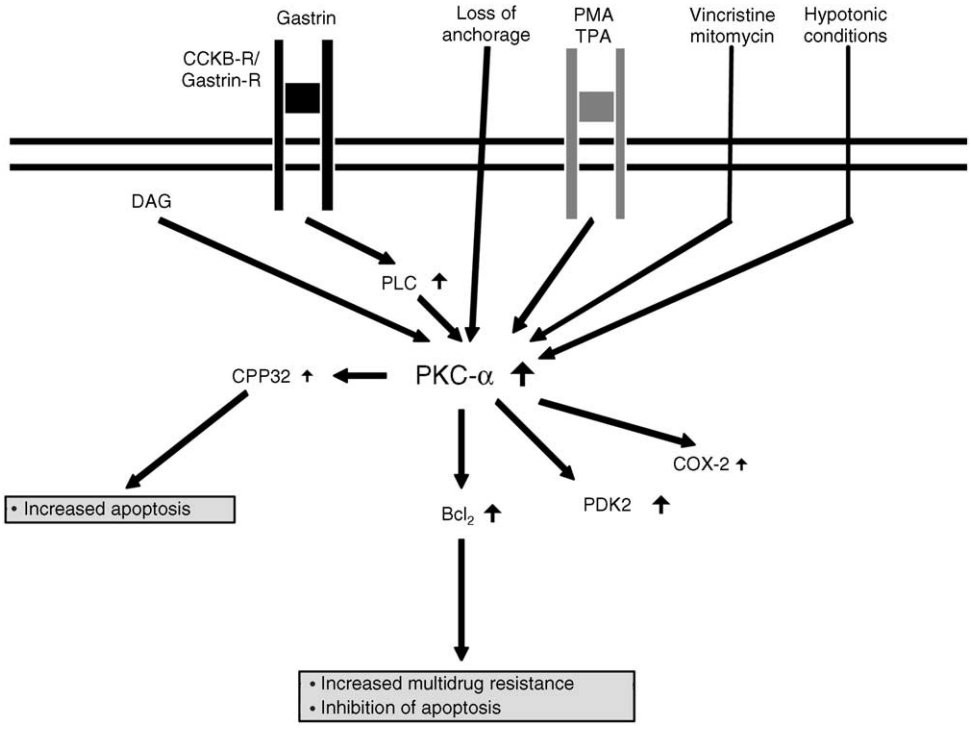

Fig. 1. PKC-α in gastric cancer. Factors that lead to protein kinase C-alpha (PKC-α) activation and PKC-α- mediated downstream gene activation in gastric cancer. Extracellular activators of PKC-α include gastrin, loss of cellular anchorage, phorbol esters (PMA/TPA), chemotherapeutics (vincristine, mitomycin) and hypotonic conditions. Intracellular activators include 1,2-diacylglycerol (DAG) and (PLC). Activated or phosphorylated PKC-α leads to increased apoptosis, multidrug resistance (MDR) and inhibition of apoptosis (grey boxes). COX-2, cyclooxygenase-2.

CPP32 [38]. Based on this observation, the role of PKC-α in mediating apoptosis may depend on whether gastric tumour cells have a high or a low metastatic potential.

In addition to regulating MDR and apoptosis, PKC-α also plays a role in gastric cancer cell proliferation. The polyphenolic phytochemical resveratol was used as a stimulus to lower PKC-α levels in KATO-III and RF-1 gastric cancer cells. This reduction in PKC-α was associated with a decreased tumour cell proliferation [42]. Similarly, in human gastric cancer AGS-B cells gastrin and DAG activated PKC-α resulting in downstream phosphorylation of protein kinase D2 (PKD2) [43]. In addition, phorbol esters, such as 3-phorbol 12-myristate 13-acetate (PMA/TPA), can induce PKC-α activation in gastric cancer cells [38,41].

2.4. Pancreatic cancer

Pancreatic tumours make up 2–3% of all malignant tumours and its incidence peaks in men of 60–80 years of age. Risk factors include smoking, chronic pancreatitis or cholecystitis, and familial risks (Lynch-syndrome II) [44]. A number of genetic abnormalities have been identified in pancreatic tumours. A point mutation at codon 12 of the K-*ras* oncogene has been described in 75–90% of pancreatic adenocarcinoma specimens and is thought to be an early event in tumour progression. Homozygous deletions have been found on the tumour suppressor genes located on 13q12 (near the *BRCA2* gene), on 9q21 (a cyclin-dependent kinase inhibitory protein), and on 18q21. A mutation in the *p53* gene located on chromosome 17p is found in 70% of pancreatic adenocarcinomas [44]. In one large study on pancreatic adenocarcinomas, gains were typically seen in chromosomes 20 and 7; in contrast to chromosomal gains, chromosomal losses were more common and involved chromosomes 18, 13, 12, 6 and 17 [45,46]. Based on these studies, additional information is needed to better understand how cytogenetic abnormalities of the PKC-α locus affect PKC-α expression in pancreatic cancer.

Increased PKC-α levels have been associated with pancreatic cancer cell proliferation. For instance, when AR4-2J pancreatoma cells were transfected with a PKC-α antisense cDNA, PKC-α expression was reduced and tumour cell growth was decreased. Reduced cell growth was proportional to PKC-α reduction [47]. Since cytokines, such as tumour necrosis factor-α (TNF-α) and interferon-α (IFN-α), have been associated with contributing to tumour cell

growth [48], their role in pancreatic cancer was examined. TNF-α and IFN-α were associated with downregulation of PKC-α in a human pancreatic adenocarcinoma (HPAC) cell line [49] and in human ductal pancreatic carcinoma cell lines, Capan 1 and Capan 2 [50]. All of these cell lines had high PKC-α expression and showed an aggressive tumour growth. Interestingly, when pancreatic cancer cell lines, AsPc1 and Capan 2, were treated with all-*trans* retinoic acid (RA), PKC-α was activated, leading to anchorage-independent growth [51]. In addition to these *in vitro* experiments, HPAC cells, which express PKC-α at high levels, were used in a xenograft animal model. In this study, tumour growth of these pancreatic cancer cells was related to PKC-α overexpression. In fact, when mice were treated with aprinocarsen decreased PKC-α expression was associated with increased survival [15].

While the above-mentioned studies show that PKC-α activation enhances pancreatic tumour growth, others have observed the opposite. For instance, pancreatic cancer cell lines treated with PMA induced PKC-α resulting in decreased phosphorylation of cyclin-dependent kinase-2 (CDK2) and retinoblastoma (Rb) protein. This decrease of both proteins led to a reduction in DNA synthesis and a reduced tumour cell growth [52]. While, this antitumour activity may be attributed to other PKC isoenzymes, additional studies are needed to better understand the conditions under which PKC-α specifically enhances or decreases tumour growth.

In an exploratory study, we compared PKC-α expression with its phosphorylated form in 17 pancreatic cancer biopsy specimens obtained from the Indiana University Tissue Bank. Western immunoblotting analyses detected PKC-α in all 17 specimens. An antibody against the phosphorylated form of PKC-α/βII (Thr 638/641; Cell Signaling Technology™) detected the activated form of PKC-α in 11 of 17 (65%) pancreatic tumour specimens (data not shown). Our data imply that PKC-α is activated and abundantly expressed in pancreatic cancers. Additional studies should be conducted to confirm these preliminary findings. If positive, such studies could provide the rationale for clinical studies with specific PKC-α inhibitors in patients with pancreatic cancer.

2.5. Hepatocellular carcinoma (HCC)

Liver tumours are one of the most common malignancies and half of these tumours occur in the gallbladder, one third in the biliary ducts; the rest are primary hepatocellular carcinomas (HCC). Incidence is geographically dependent, with China and Korea having the highest incidence rates. Because of the rising incidence of HCV in industrialised countries, HCC is expected to increase in North America and Europe [53]. Cytogenic analyses of HCC show complex cytogenic abnormalities with losses of chromosomes 13q, 16q and 17p [54]. To date, few studies found chromosomal 17q gains in HCC and thus amplification of the *PKC-α* gene may be uncommon [55].

PKC-α has been associated with tumour cell proliferation and various stimuli can lead to increased PKC-α activation in the liver (Fig. 2). Importantly, in rats, prolonged choline deficiency increased PKC-α and PKC-δ expression, which resulted in a reduction of glycogen synthase. This PKC-dependent dysregulation was associated with spontaneous hepatocarcinogenesis [56]. In addition to this sustained PKC-α activation, pro-inflammatory cytokines, such as TNF-α and IL-1α [48], have also been associated with PKC-α activation. For example, using antisense oligonucleotide blocking PKC-α, IL-1α was recognised to specifically phosphorylate PKC-α, which in turn activated IκBα [57]. Thus, PKC-α appears to play not only a direct role in hepatocarcinogenesis, but also an indirect role by promoting an adverse immune environment.

In addition to pro-inflammatory cytokines, growth factors have been implicated in the activation of PKC-α. Activating rat hepatoma cells with PMA tumour cells proliferated in a PKC-α-dependent way, as confirmed by experiments with antisense oligonucleotides (ASO) against PKC-α [58]. Furthermore, in Hep3B cells, insulin phosphorylated PKC-α, which subsequently activated early-growth-regulatory-1 (*egr-1*) gene expression. This insulin-induced activation increased cell tumour proliferation [59]. Oestrogen and 17-β-oestradiol by activation of inositol trisphosphate (IP3) can also induce the phosphorylation of PKC-α in HepG2 cells. This activation caused phosphorylation of the cytosolic oestrogen receptor (ER) and subsequent cell proliferation [60,61].

Phosphorylation of PKC-α can lead to various downstream signals (Fig. 2). For example, PMA-induced PKC-α expression led to erythropoetin (EPO) production in HepG2 cells [62]. Similarly, hypoxia induced PKC-α-dependent EPO production in Hep3B cells, as confirmed by experiments with ASO against PKC-α [63]. Lastly, in rat liver epithelial tumour cells, increased PKC-α can activate Nuclear Factor (κB) (NFκB) and Bcl_2 resulting in tumour cell proliferation and reduction of tumour cell apoptosis, respectively [64].

In contrast to these *in vitro* studies, one study measured PKC-α levels in tumour biopsy specimens from patients with HCC. This study found that the level of membrane-bound PKC-α was significantly lower in HCC than that in the adjacent normal tissue. In addition, PKC-α expression negatively correlated with tumour size [65]. These observations implied that PKC-

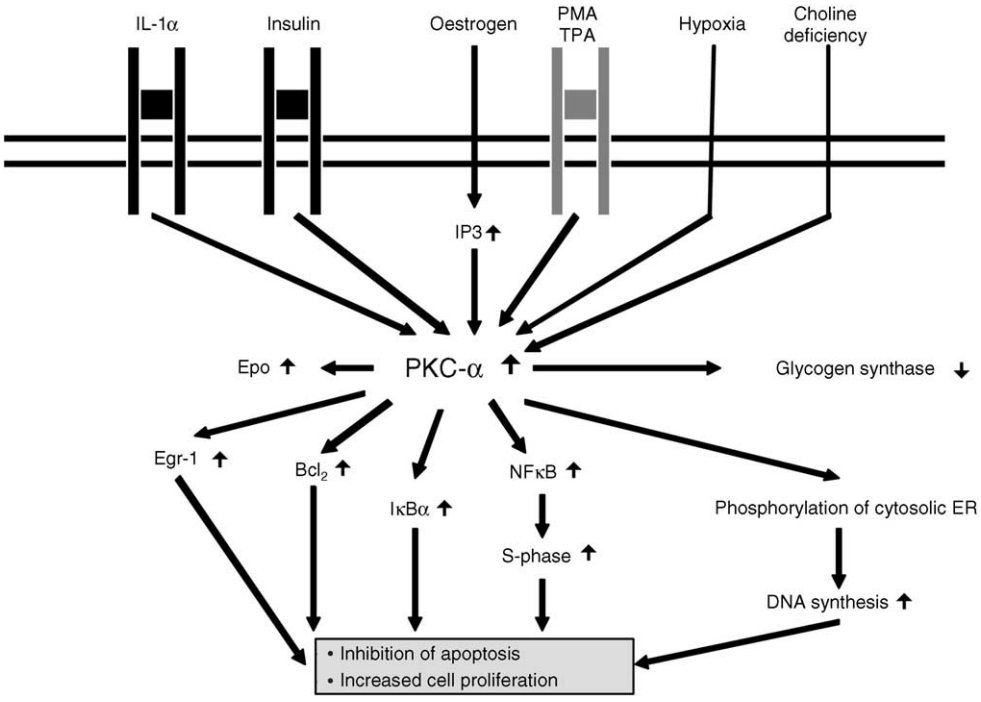

Fig. 2. PKC-α in hepatic cancer. Factors that lead to PKC-α activation and PKC-α-mediated downstream gene activation in hepatic cancer. Extracellular activators of PKC-α include interleukin-1α (IL-1α), insulin, oestrogen, phorbol esters (PMA, TPA), hypoxia and choline deficiency. Intracellular activators include IP3. Activated or phosphorylated PKC-α leads to increased erythropoetin secretion, decreased glycogen synthesis as well as to inhibition of apoptosis and increased tumour cell proliferation (grey box).NFκB, Nuclear Factor κB; ER, oestrogen receptor; IP3, Inositol triphosphate.

α plays a role in early tumorigenesis of HCC and not in late stage HCC. Since no information was available on PKC-α and its phosphorylated form in HCC, we obtained five hepatic tumour specimens from the Indiana University Tumor Tissue Bank and performed Western immunoblotting. We found detectable PKC-α in all samples, while its phosphorylated form was not detected (data not shown). Although this study is exploratory, due to its sample size, our findings would support previous studies in patients with HCC [65]. Taking all these studies together, it is unlikely that patients with HCC will benefit from inhibition of PKC-α.

2.6. Colorectal cancer

Approximately 10% of all solid tumours are colorectal cancers. Frequent cytogenetic changes associated with colorectal cancer include rearrangement of chromosome 17, leading to the loss of 17p, which contains the *p53* gene locus, and loss of chromosome 18 [66]. The most common numerical abnormalities are, in order of decreasing frequency, gains of chromosomes 7, 13, 20, and Y and losses of chromosomes 18, Y, 14, and 15 [67]. Chromosome 17p is lost at a high frequency in colorectal cancers. Indeed, the most important independent variable reported in the prognosis of stage IB/II colorectal carcinomas was tumour stage and high-grade p53 expression in the tumour cells. Chromosome 17 aneusomy was an independent risk factor for tumour relapse/progression, but not for survival. Because these cytogenetic changes were not found in all carcinomas, alternative pathogenetic pathways may exist in colorectal carcinogenesis [68]. Such alternative pathways may include loci of signalling proteins, such as PKC-α. However, the impact of chromosome 17q gains in colorectal tumours requires additional research. One study found that although there are partial gains of chromosome arms 17q, 8q, and 13q, the most common partial losses affected chromosome arms 17p, 1p, 8p, and 13p [67]. Based on this study, the PKC-α locus on chromosome 17q may not be consistently altered in colorectal cancer and this may explain why PKC-α may on the one hand promote tumour growth and on the other, have anti-tumour activities (Fig. 3).

For instance, in normal colon tissue of rats and humans, colon mucosa contained PKC-α, PKC-δ, PKC-ε and PKC-ζ which are consistently expressed. In general, PKC isoenzyme protein expression was greater at the top of the crypt axis and was associated with cells having acquired a differentiated phenotype [69]. This observation that PKC may play a role in the differentiation process of colon mucosa cells is also seen in CaCo-2 cells after their transfection with ASO against PKC-α. In these cells, the reduced levels of PKC-α

resulted in a decrease in cell differentiation and proliferation [70]. Further evidence that PKC-α plays a role in tumour cell proliferation is seen in CaCo2 cells after they are activated with $1,25(OH)_2D_3$. Under this particular condition, CaCo2 cells show increased apoptosis and differentiation, as well as decreased proliferation. This PKC-α effect is mediated by an activation of ERK2 and JNK1 and subsequent activation of AP-1 and c-jun increasing alkaline phosphatase [71]. Moreover, in CaCo2 cells, HGF activated upon binding to its receptor HGF-R (c-Met) PKC-α, thus inducing differentiation as measured by increased expression of enterocytic differentiation markers (e.g. alkaline phosphatase, sucrose-isomatase activity, E-cadherin, villin, F-actin and actin fibre reorganisation) [72]. TGF-β_1 can also function as a differentiation factor by activating PKC-α and inhibiting cellular proliferation. An ASO directed to block PKC-α renders human colon cancer cells unresponsive to TGF-β_1 [73]. TGF-β_1-induced PKC-α activation increases not only CEA [73], but also fibronectin and laminin expression [74]. Both of these matrix adhesion molecules and the intracellular adhesion molecule, CEA, are thought to reduce proliferation. In addition to these molecules, TGF-β_1 induces PKC-α-dependent expression of E-cadherin and the undercoat-associated proteins, α- and β-catenin, in Moser colon cancer cells [75]. Consistent with these *in vitro* observations, reduced levels of PKC, including PKC-α, PKC-γ and PKC-ζ, were found in colorectal cancer biopsies of patients with advanced cancer [76]. Similarly, patients with adenomas of the colon had lower PKC-α levels compared with normal tissues [77]. Moreover, an anti-tumour effect of PKC-α was also seen murine tumour models. Intestinal HT-29 M6 cells expressing activated PKC-α were implanted into athymic mice showing a slow tumour growth. Especially HT-29 M6 cells with the highest PKC-α expression showed no subcutaneous formation of tumours. This study implied that PKC-α activity inversely modulated invasion and growth of intestinal tumour cells [78]. Thus, it is possible that under currently unknown conditions, PKC-α activation can contribute to a less aggressive tumour phenotype.

In contrast to the above-mentioned studies, PKC-α can have the opposite effect and promote tumour growth. For example, using an ASO against PKC-α,

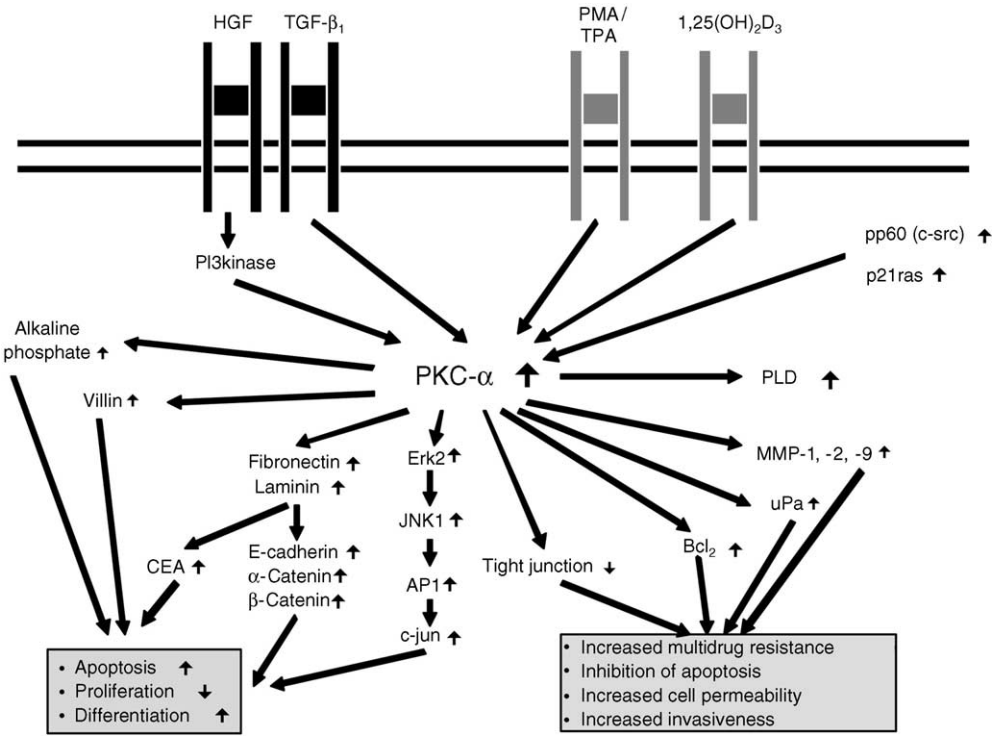

Fig. 3. PKC-α in colorectal cancer. Factors that lead to PKC-α activation and PKC-α-mediated downstream gene activation in colorectal cancer. Extracellular activators of PKC-α include HGF, TGF-β_1, phorbol esters (PMA/TPA) and vitamin D3 ($1,25(OH_2D_3)$). Intracellular activators include PI3 kinase, c-src and p21ras. Activated or phosphorylated PKC-α can lead to two different effects on colorectal cancer cells, one being anti-tumour (on the left side of the cartoon) and the other promoting tumour growth (on the right side of the cartoon). Anti-tumour activity of PKC-α is characterised by increased alkaline phosphatase, villin, carcinoembryonic antigen (CEA) and other cell adhesion molecules. This results in increased tumour cell apoptosis, decreased tumour cell proliferation and increased tumour cell differentiation (grey box on the left). Tumour promoting activity of PKC-α is characterised by increased PLD and a number of anti-apoptotic molecules (e.g., Bcl_2) leading to increased multi-drug resistance, inhibition of apoptosis, increased cell permeability and invasiveness (grey box). uPA, urokinase plasminogen activator; PLD, phospholipase D. TGF-β_1-, transforming growth factor-β_1; MMP, matrix metalloproteinase.

migration of six different human adenocarcinoma cell lines was inhibited and associated with low E-cadherin expression [79]. Especially in the highly metastatic variant (L-10) of the human colon adenocarcinoma cell line RCM-1, PKC-α expression was associated with high tumour cell motility and invasiveness. This was specific to the function of PKC-α since an ASO against PKC-α arrested the invasiveness of these colon cancer cells and an additional blocking agent was required to reverse the colon cancer phenotype [80].

Although speculative, there are some studies that might explain the opposite function of PKC-α in colorectal cancer. While cancer cell lines may have inherently different growth patterns impacting the regulation of PKC-α-dependent mechanisms, growth factors may contribute to a differential activation of signalling pathways. For example, HGF can, on the one hand, be associated with increased differentiation of CaCo-2 cells [72], and, on the other hand, it can contribute to increased tumour motility and invasiveness. This HGF-induced invasive phenotype was associated with a PKC-α-dependent production of proteases, such as metalloproteinase-1 (MMP-1), MMP-2, MMP-9 and urokinase plasminogen activator (uPA) [81]. In addition to growth factors, PKC-α-dependent colon cancer cell proliferation may be influenced by loss of tight junctions. Short- or long-term activation of PKC-α led to a reduction of tight junctions and subsequent paracellular leakiness allowing cells to detach and to migrate [82]. Such a reduction of tight junctions was associated with epidermal growth factor (EGF) accessing basal-lateral cells and further activating PKC-α [83]. These studies underline the assumption that tight junctional leakiness is a late event in epithelial carcinogenesis and allows growth factors to enter intercellular and interstitial fluid spaces. Proto-oncogenes, such as *ras* and *src*, are also associated with activation of PKC-α in human colorectal cancers [84]. In CaCo-2 human colon cancer cells, p21ras and pp60 (c-src) activated PKC-α and subsequently increased tumorigenicity. Furthermore, when alpha-tocopheryl-succinate (α-TOS) was administered to treat colon cancer xenografts implanted in athymic mice, PKC-α was activated and found to phosphorylate Bcl_2. In this model, antitumour activity was obtained when mice were treated with an ASO blocking PKC-α [85]. PKC-α also has an influence on inflammatory mediators in colorectal cancer. For example, PKC-α has been associated with activation of phospholipase D (PLD) as studies with an ASO blocking PKC-α have suggested [86,87]. In addition to enhancing tumour growth, PKC-α overexpression is associated with increased multi-drug resistance (MDR) in colorectal cancer. A study using P-glycoprotein-devoid colon cancer cells suggested MDR is not necessarily linked to *mdr* genes. After PKC-α expression was induced in P-glycoprotein-devoid colon cells via PMA, doxorubicin and vincristine uptake was reduced while 5-fluorouracil (5-FU) was not affected [88]. The particular characteristics of this MDR was also seen in other colon cancer cells, such as Moser, SW 480 and HT29 cells, and was reversed after administration of an ASO blocking PKC-α. Once PKC-α was inhibited, Moser cells became susceptible to mitomycin C, 5-FU and vincristine [89]. Finally, in a cell line established from patients with metastatic colon cancer and high endogenous PKC-α expression (KM12L4a), the activation of PKC-α was associated with increased MDR [22]. In contrast to these observations, LoVo human colon adenocarcinoma cells seem to require PKC-α as part of their mechanism to avoid MDR. Since this experiment was performed with a PKC-α unspecific inhibitor (Go6976), the specific role of PKC-α and its contribution to MDR remain to be determined [90].

While all the above-mentioned observations were made mainly *in vitro*, we wanted to test the expression pattern in tumour biopsies. We obtained 33 tumour specimen of patients with colorectal tumours from the Indiana University Tumor Tissue Bank. Using Western immunoblotting we detected PKC-α in 16 of 22 (73%) samples and the phosphorylated form of PKC-α (Phospho PKC-α/βII Thr 638/641;Cell Signaling Technology™) in 24 of 33 (73%) specimens (data not shown). In addition to Western immunoblotting, we used a novel technique that allows the quantitative mRNA analyses of gene expression from paraffin-embedded tumour specimens [91]. We obtained an additional 22 tumour specimens from patients with colorectal cancer from the Indiana University Tumor Tissue Bank. Based on our earlier assessments (data not shown), the Dannenberg tumour profile (DTP) was high (DTP = 5.0) in all of these tumour specimens (Fig. 4a). We also correlated *PKC-α* gene expression with the gene expression of factors found to be activated downstream of PKC-α (Fig. 3). Due to their role in mediating tumour cell migration, we looked at *integrin* β_3 [92] and ezrin [93]. Furthermore, we determined *mdr-1* gene expression, because of its association with colorectal cancer [94]. Finally, we looked at the apoptosis marker bcl_2, which is especially upregulated in advanced colorectal cancers [95]. Although the sample size was small, there was a trend for *PKC-α* expression to correlate positively with *mdr-1*, bcl_2, *ezrin*, and *integrin-*β_3 (Fig. 4b–e). These observations confirm some of the findings obtained from cell line experiments and may allow the development of future PKC-α-related gene expression profiles as a tool in the clinical development of PKC-α inhibitors.

2.7. Clinical studies

We briefly review here the clinical trial experience of a specific inhibitor to PKC-α, aprinocarsen. This phosphorothioate antisense oligonucleotide (ASO) is

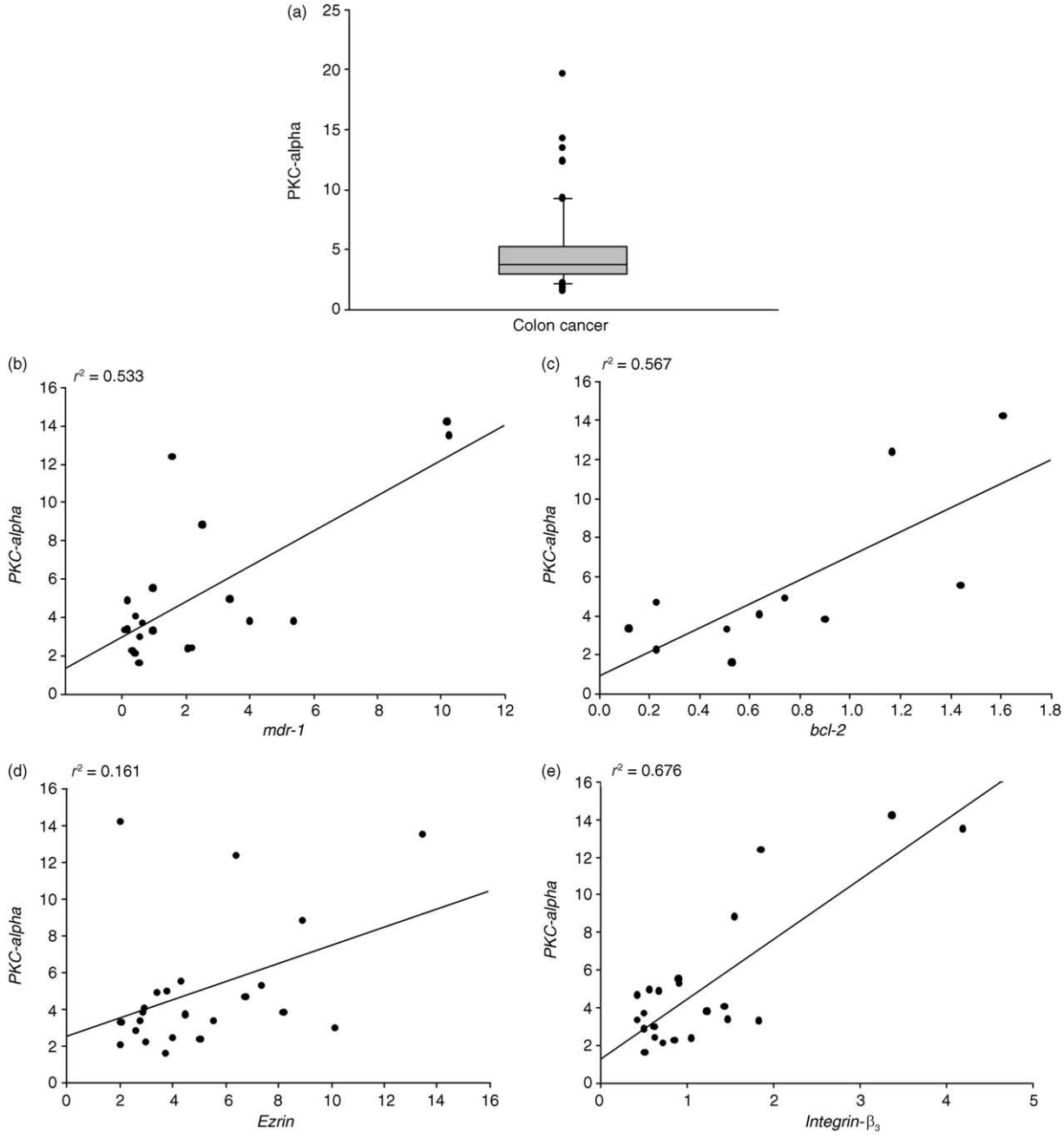

Fig. 4. Quantitative mRNA gene expression analysis of PKC-α expression in 22 colorectal cancer specimens using a previously published method [91]. Panel a. PKC-α expression is present in all tumour specimens. Mean is at 5.0 Dannenberg Tumor Profile (DTP). Distribution of PKC-α expression in 22 colorectal cancer specimens is represented as a box plot graph. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. In addition, outlying points are shown. Panels 4b–e: *PKC-α* gene expression values are depicted as a scatter-plot and positively correlate with *mdr-1* (panel b), bcl_2 (panel c), *ezrin* (panel d), and *integrin-*β_3 (panel e) gene expression. On all panels, *PKC-α* gene expression values are given on the *y*-axis, and correlative gene expression values for the specific protein described is given on the *x*-axis. The regression line is inserted to facilitate understanding of the correlation coefficient, which is given on the upper left corner of the graph.

directed to inhibit the mRNA for *PKC-α* and by binding to it allows its enzymatic degradation by RNase H, thus blocking the protein synthesis of PKC-α [96]. Aprinocarsen has been tested in two trials, CS04 and CS05, in patients with colorectal cancer [97,98].

In study CS05, aprinocarsen was given as a single-agent as part of a 21-day continuous infusion at 2 mg/kg/day in 17 patients. Of these 17 patients, 4 patients had stable disease as the best response with a median duration of 3.4 months (range 1.8–8.5 months) [98]. In study CS04, aprinocarsen was given

as a 21-day continuous infusion in 15 patients with advanced cancer in combination with 5-FU and leucovorin at doses of 1–2 mg/kg/day. Of these 15 patients, 10 had colorectal cancer. During this study, 2 patients showed partial responses and 4 patients had stable disease [97].

PKC-α levels were not determined at baseline or at the end of the aprinocarsen infusion in any of these studies. Thus, it is difficult to judge the effect of aprinocarsen in these patients.

3. Conclusion

Our review on the role of PKC-α in the tumorigenesis of gastrointestinal cancers suggests that additional histopathological studies combined with the collection of clinical data are necessary prior to determining which patients may benefit from a specific inhibition of PKC-α. Because most of the studies on PKC-α have been *in vitro*, it currently remains challenging to estimate which tumour type is likely to respond to a PKC-α inhibition. However, oesophageal and pancreatic cancers appear to be tumour types where a tumour response is likely. In colorectal cancers, PKC-α seems to be involved in a more aggressive phenotype [22], but under as yet to be identified conditions, PKC-α can have an antitumour effect. Finally, HCC appear not to express activated PKC-α and future studies are needed to understand how PKC-α contributes to tumour progression in HCC.

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