Pathol. Oncol. Res. (2010) 16:385–391
DOI 10.1007/s12253-009-9228-z

Expression of Protein Kinase C Family in Human Hepatocellular Carcinoma

Hsiu-Chin Lu · Fen-Pi Chou · Kun-Tu Yeh · Ya-Sian Chang · Nicholas C. Hsu · Jan-Gowth Chang

Received: 8 July 2009 / Accepted: 10 November 2009 / Published online: 1 December 2009

Abstract Protein kinase Cs (PKCs) play important roles in signal transduction, cell regulation, and tumor formation. In the present study, we analyzed the expression of PKCs in human hepatocellular carcinoma (HCC) tissues and explored their roles in the development of HCC. Real-time quantitative PCR and immunohistochemistry showed that PKCβ and PKCθ were down-regulated in HCC tissues. Reduced expression of PKCθ is well correlated with the grade of cancer cells (p=0.009), and the down-regulated expression of PKCβII is associated with HBV infection (p=0.035). Our findings suggest particular roles of the two PKC isoenzymes in the hepatocarcinogenesis of human HCC.

Keywords Protein kinase C · Human hepatocellular carcinoma

Electronic supplementary material The online version of this article (doi:10.1007/s12253-009-9228-z) contains supplementary material, which is available to authorized users.

H.-C. Lu · Y.-S. Chang · N. C. Hsu · J.-G. Chang
Department of Laboratory Medicine,
Kaohsiung Medical University Hospital,
Institute of Clinical Research, College of Medicine,
Kaohsiung Medical University,
Kaohsiung, Taiwan

H.-C. Lu · F.-P. Chou
Institute of Biochemistry and Biotechnology,
Chung-Shan Medical University,
Taichung, Taiwan

K.-T. Yeh
Department of Pathology, Changhua Christian Hospital,
Changhua, Taiwan

J.-G. Chang
Center for Excellence in Environmental Medicine,
Kaohsiung Medical University,
Kaohsiung, Taiwan

J.-G. Chang (✉)
Department of Laboratory Medicine,
Kaohsiung Medical University Hospital,
Kaohsiung, Taiwan
e-mail: jgchang@ms.kmuh.org.tw

Introduction

Hepatocellular carcinoma (HCC) is commonly associated with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, with chronic exposure to aflatoxin B, or with complications as a consequence of alcoholic cirrhosis [1–5]. The oncogenesis of HCC is thought to be a multiple-step process which includes inactivation of tumor suppressor genes, activation of oncogenes, or disturbance of other genes that ultimately lead to the formation of tumor [1, 6–9].

Protein Kinase C (PKC) family consists of at least 12 seine-threonine kinases which are classified into three groups: classical (α, β, and γ), novel (δ, ε, η, and θ) and atypical (μ, ξ, and ι). Activation of classical enzymes is dependent on calcium and diacylglycerol (DAG), novel enzymes are activated by diacylglycerol. Atypical enzymes are not activated by calcium or DAG, but they may be activated by other PKCs [10–13]. Sustained activation of PKCs induces long-term effects including proliferation, differentiation, apoptosis, migration and tumorigenesis [14–21]. PKC isoenzymes are ubiquitously expressed in tissues. PKC α, β, and δ are the most abundant isoenzymes in various tissues [22]. Activation of different PKC isoenzymes has been shown to result in different cellular response, and there is an extensive cross-talk with different isoenzymes, and the overall response is dependent on presence or activity of the other isoenzymes in particular cell type [18, 21].

PKC plays a major role in intracellular signal transduction and is thought to be involved in cancer biology. Moreover, the biological effects of PKC seem to be tumor-specific rather than unique. PKCα is overexpressed in high grade urinary bladder, prostate, and endometrial cancers, but low grade tumors and normal epithelia of the respective organs show significantly reduced expression [23–28]. In contrast, breast, colon, hepatocellular and basal cell cancers show down-reguled PKCα expression [29–33]. PKCβ expression has been shown to be up-regulated in colon and prostate cancers [24, 30], but down-regulated in bladder cancer [25, 26, 28]. In this study, we analyzed the expression of 10 PKCs in HCC and explore their clinical significance.

Materials and Methods

Samples

Fifty-five histologically confirmed resected HCCs and paired non-cancerous tissue samples were obtained on protocols approved by the Institutional Review Board of Changhua Christian Hospital. The age of the patients ranged from 24 to 77 years with a mean of 56.8 years. All patients were staged according to the 2002 American Joint Committee on Cancer staging system. The Edmondson-Steiner grading system was used [34]. Pathologically, all tumors were HCC with 12 cases at grade I, 28 cases at grade II and 15 cases at grade III. Slides from tumors were reviewed by two pathologists to define the histological grading. Tissues were frozen immediately after surgical resection and stored in liquid nitrogen until extraction of DNA or RNA. DNA extraction was performed as previously described [35]. Total RNA was extracted using a commercial kit (RNA-Bee™, Tel-Test, Inc., Texas, USA), and stored in −70°C deep freezer either as a pellet in ethanol or solubilized in RNase-free water.

Real-Time Quantitative RT-PCR (qRT-PCR) Analysis

The mRNA sequences of the 10 PKC genes were evaluated for the purpose of designing specific forward and reverse primers and specific probes with the aid of the Primer Express Software (Roche, USA). The probes were synthesized and labeled with appropriate fluorescent dyes (Roche). Sequences of the forward and reverse primers for 10 PKC genes are listed in the Supplement 1. We used *HPRT* gene as internal RNA control for RT-PCR. The expression levels of the PKC genes were normalized to the endogenous *HPRT* reference to obtain the relative threshold cycle (ΔCt) which was in turn related to the ΔCt of the paired non-cancerous tissue to obtain the relative expression level ($2^{-\Delta\Delta Ct}$) of the PKC gene.

Reverse transcription was performed in a final volume of 25 μL containing 2 μg RNA, 0.5 μg random primers (10 mers), 2 mM dNTPs, 25 U RNasin (Promega, Madison, WI, USA), 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, and 200 U Moloney Murine Leukemia Virus reverse transcriptase (Promega). The reaction was first denatured for 5 min at 70°C and incubated at 37°C for 60 min, then stopped by heat inactivation at 95°C for 5 min. Real-time quantitative PCR was performed as described [35]. $2^{-\Delta\Delta Ct}$ indicates the ratio of concentration of PKC mRNA of tumor/normal.

Analysis of Alternative Splicing of Pre-mRNA of PKCβ in HCC

The RT-PCR analysis were performed as described [36]. The sets of primers used for screening alternative splicing of PKCβ were shown in Supplement 2. The PCR was performed with a denaturing step at 94°C for 5 min , then 30 s at 94°C, 30 s at Tm of primers , and 1 min at 72°C for 35 cycles , followed by a final 5 min at 72°C. The PCR products were separated on 2.5% agarose gel and the intensity of the PCR products were analyzed by LabWorks Image Acquisition and Analysis Software (UVP BioImaging Systems).

Analyses of PKC Isoenzymes Expression by Immunohistochemistry

The percentage of immunoreactivity was scored as 0 to +2. Immunoreactivity in <10% of cells was considered as negative expression (0), 10–50% (+1), and >50% (+2). Five-to-ten fields were examined for each section, and at least 1000 cells were scored. Investigator-bias was avoided by two investigators independently scoring coded sections. The staining intensity of non-tumorous hepatocyte was considered as an internal positive control and 1+ staining. Briefly, paraffin-embedded HCC and paired non-cancerous tissue sections (4 μm) on poly-1-lysine coated slides were deparaffinized. After treatment of 3 % H_2O_2 in methanol, the sections were hydrated with gradient alcohol and PBS, incubated in 10 mM citrate buffer and, finally, heated at 100°C for 20 min in PBS. After incubation with the anti-PKCβI, PKCβII, or PKCθ antibody (all purchased from Santa Cruz Biotechnology, Santa Cruz, CA) for 20 min at room temperature, slides were incubated with a horseradish peroxidase (HRP)/Fab polymer conjugate for another 30 min after being thoroughly washed three times with PBS. The sites of peroxidase activity were visualized using 3, 3'-diamino-benzidine tetrahydrochloride as a substrate and hematoxylin as the counter stain. The paired non-cancerous liver tissues were used as positive controls for the PKC protein. Negative controls were defined as tissues of negative immunoreactivity with PBS substituting the anti- PKC antibody in IHC.

Table 1 Expression of 10 PKC genes in 55 pairs of HCC and their paired nearby normal tissues by qRT-PCR

Gene name	Tumor $^{\triangle}$Ct (PKC-HPRT1)	Normal $^{\triangle}$Ct (PKC-HPRT1)	-$^{\triangle\triangle}$Ct -(tumor $^{\triangle}$Ct-normal $^{\triangle}$Ct)	2-$^{\triangle\triangle}$Ct	*P* value
PKCα	−2.157	−2.049	0.108	1.078	0.812
PKCβ	2.875	0.981	−1.894	0.269	1.29E-05
PKCδ	−0.163	−0.049	0.114	1.082	0.729
PKCε	0.449	−0.003	−0.451	0.731	0.229
PKCθ	3.288	1.972	−1.315	0.402	0.014
PKCζ	−0.099	−0.900	−0.801	0.574	0.090
PKCμ	1.940	1.348	−0.591	0.664	0.138
PKCν	1.074	0.978	−0.100	0936	0.812
PKCι	6.78	6.16	−0.62	0.65	0.06
PKCγ	11.32	10.58	−0.74	0.6	0.3

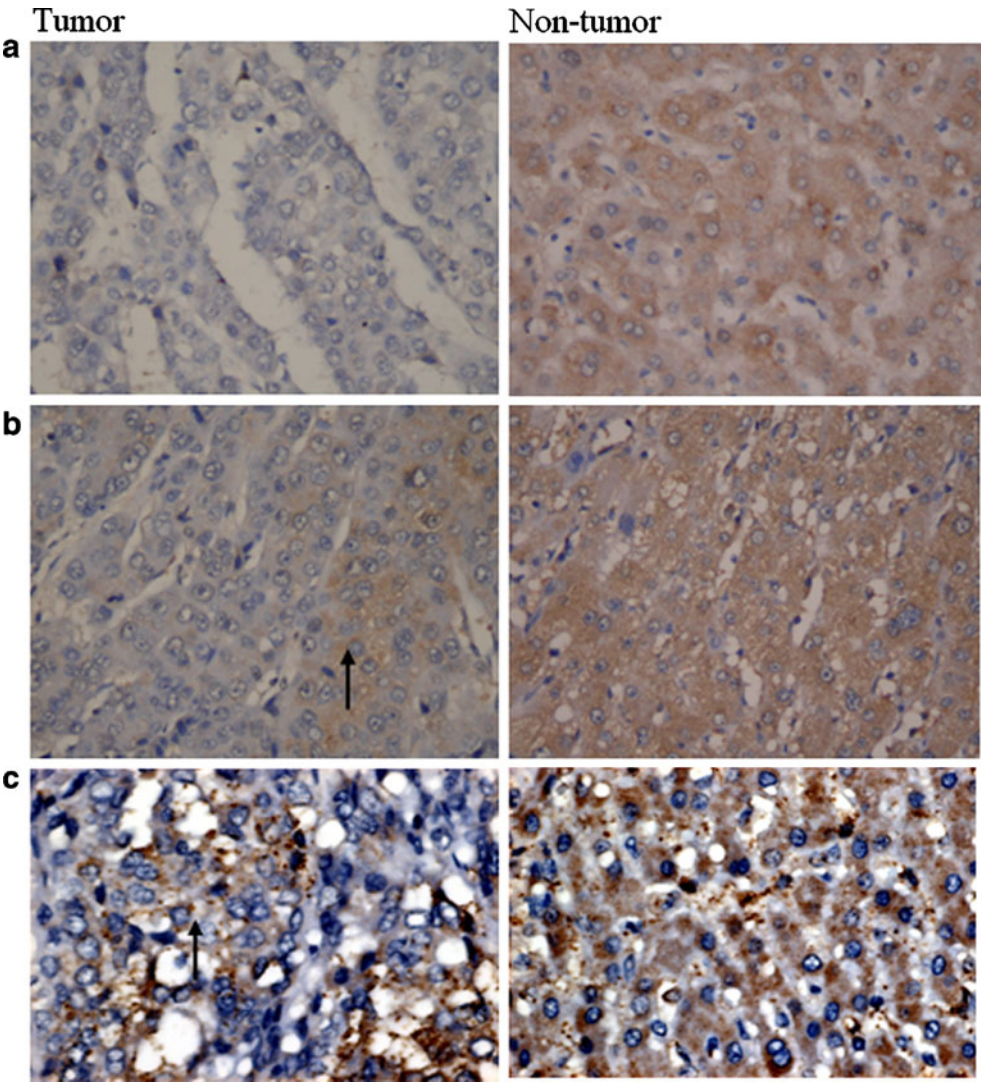

Fig. 1 Immunohistochemical analysis of **a** PKCβI, **b** PKCβII, and **c** PKCθ in HCC. Representative figures of heterogeneous expression (*arrow*) in the tumor part and homogeneous expression in the paired non-tumor part are shown

Table 2 Correlation between clinicopathological features and the expression of PKCβII and PKCθ in HCC

		PKCβII				PKCθ			
		T < N	T ≧ N	Total	*P*-value	T < N	T ≧ N	Total	*P*-value
Gender	Female	7	5	12	0.751	12	0	12	0.319
	Male	22	21	43		36	6	42	
Tumor size	<=2 cm	3	4	7	0.696	5	2	7	0.169
	>2 cm	26	22	48		43	4	47	
Grade	Well	4	1	5	0.351	2	3	5	0.009
	M + P	22	23	45		42	3	45	
Stage	I, II	18	10	28	0.173	25	2	27	0.662
	III, IV	11	14	25		22	3	25	
Survival	<=2 years	10	9	19	1.000	18	1	19	0.408
	>2 years	19	17	36		30	5	35	
HBV infection	–	3	8	11	0.035	10	1	11	1.000
	+	24	12	36		32	3	35	
HCV infection	–	16	13	29	1.000	27	2	29	0.162
	+	7	7	14		10	3	13	
Cirrhosis	–	9	8	17	1.000	17	0	17	0.286
	+	15	14	29		26	3	29	
Smoker	–	19	16	35	0.734	32	2	34	1.000
	+	5	6	11		11	0	11	
Drink	–	20	15	35	0.217	32	2	34	1.000
	+	5	9	14		13	1	14	
α-fetoprotein	<20	15	13	28	1.000	22	5	27	0.193
	>20	13	12	25		24	1	25	

P-value by Chi-square test or fisher's exact test when appropriated

Statistics

Comparisons between the RNA expression levels of the ten PKC genes in HCC and non-tumor tissues were analyzed by *t*-test run on SPSS for Windows Release 9.0 (SPSS, Chicago, IL).

Results

Expression of 10 PKC Genes in HCC

We used qRT-PCR to analyze the expression of 10 PKC genes in 55 pairs of HCC and their paired nearby non-cancerous tissues. The results are shown in the Table 1. We found that PKCβ and PKCθ are down-regulated in HCC in comparison with their paired nearby non-HCC tissues. There are no statistical difference for the expression of PKCα, PKCδ, PKCε, PKCζ, PKCμ, PKCι, PKCν, and PKCγ between HCC and their paired non-cancerous tissues.

RT-PCR Analysis of Splicing Variants of PKCβ

We analyzed the expression of the splicing variants of PKCβ in HCCs and paired non-HCC tissues. The results showed that PKCβII variant form was found in 21/55 of non-HCC and 11/55 cancerous tissues.

Immunohistochemical Analysis of 2 PKC Isoforms in HCC

In order to confirm the results of qRT-PCR, we used the immumohistochemical staining to analyze the expression of PKCβ and PKCθ. The results show significant reduction of PKCβ and PKCθ in HCC cancerous cells in comparison with nearby non-cancerous cells, compatible with the results of qRT-PCR (Fig. 1). The normal tissues show homogenous expression pattern in liver cells in comparison with the heterogeneous expression feature of HCC cells which includes expressed and decreased or unexpressed PKCθ or PKCβ proteins in different cancerous cells in the tumor tissue (Fig. 1). We compare the clinicopathological data with the expression of PKCβI, PKCβII, and PKCθ,

and find that the down-requlated expression of PKCθ is well correlated with the grade of cancerous cells (P=0.009), and the reduced expression of PKCβII is associated with HBV infection (P=0.035) (Table 2).

Discussion

Within the cell, each PKC isoform mediates distinct functions including regulation of mitogenesis cell cycle, apoptosis and gene expression [13–21]. PKC isoforms also play an important role in neoplastic transformation, the growth and metastasis of tumor in a variety of tissues [15–18, 21, 22, 26, 37–40]. In HCC cells, aberrant levels of PKCs are suggested to contribute to liver neoplasia and transformation [41–43]. PKCα has been associated with tumor cell proliferation and various stimuli can lead to increased PKCα activation in the liver [27, 41–43]. PKCθ has been demonstrated with proliferation of aortic smooth muscle cells and colonic cells [44, 45], and it plays an essential and important function for T-cell and immunological synapse [46, 47]. PKCθ regulates AKT signaling pathway and KIT expression [48]. Over-expression of PKCθ may result in cancer development [48–52]. Inactivation of PKCθ leads to increased susceptibility to obesity and dietary insulin resistance in mice [53, 54], and impaired anti-leukemic immune response [55]. We find that unlike other types of cancer, PKCθ in HCC is downregulated [48–52]. These discrepancy maybe due to increase susceptibility of insulin resistance which favors the survival of HCC cells. The down-expression of PKCθ is well correlated with poorer grade of HCC cells.

Over-expression of PKCβII results in poor prognosis in nodal diffuse large B-cell lymphoma [56]. In our study, we are unable to establish an association between prognosis and the expression of PKCβII, but we found a correlation between HBV infection and the downregulation of PKCβII. Bazarsky et al demonstrated that persistent measles infection affects the expression of PKCα, ε and ζ in neuroblastoma cell lines [57]. We suggest that HBV infection may influence the expression of PKCβII which play a role in the early phase of oncogenesis but not at late stage. Similar results have been found in transitional cell carcinoma of bladder [58].

Despite of considerable heterogeneity in the expression of PKCs, correlations could be established between certain PKC expression patterns and pathological or virological features of HCC. Individual genetic variations cannot account for the heterogeneity of PKC expression pattern, because cancer and non-cancerous tissues from the same patients were examined in this study. We reasoned that the heterogeneity of PKCs is a direct result of the complexity of hepatocarcinogenesis in human HCC.

There is accumulating evidence against the specificity of alpha-fetoprotein (AFP) in making a diagnosis of HCC because AFP can also be produced by other malignancies, such as gastric, pancreatic, colon, bladder, and lung cancers [59–61]. Moreover, given the high heterogeneity of HCC, AFP is normal in many HCC patients [62]. Because of its limited sensitivity and specificity, AFP alone is not considered useful for the surveillance of HCC [63]. More than 50% of our patients in this study have normal AFP which makes AFP a poor indicator of HCC (Table 2).

In conclusion, our results indicate that aberrant signal transduction via PKC may occur during the formation of liver cancer. The fact that down-regulated expression of PKCβII and PKCθ were correlated with HBV infection and the grade of cancer cells, respectively, suggest particular roles of the two PKC isoenzymes in the development of human HCC.

References

1. El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterol 132:2557–2576
2. Marrero CR, Marrero JA (2007) Viral hepatitis and hepatocellular carcinoma. Arch Med Res 38:612–620
3. McGlynn KA, London WT (2005) Epidemiology and natural history of hepatocellular carcinoma. Best Pract Res Clin Gastroenterol 19:3–23
4. Morgan TR, Mandayam S, Jamal MM (2004) Alcohol and hepatocellular carcinoma. Gastroenterol 127:S87–96
5. Seeff LB, Hoofnagle JH (2006) Epidemiology of hepatocellular carcinoma in areas of low hepatitis B and hepatitis C endemicity. Oncogene 25:3771–3777
6. Feitelson MA, Sun B, Satiroglu Tufan NL et al (2002) Genetic mechanisms of hepatocarcinogenesis. Oncogene 21:2593–2604
7. Laurent-Puig P, Zucman-Rossi J (2006) Genetics of hepatocellular tumors. Oncogene 25:3778–3786
8. Thorgeirsson SS, Grisham JW (2002) Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 31:339–346
9. Villanueva A, Newell P, Chiang DY et al (2007) Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis 27:55–76
10. Mellor H, Parker PJ (1998) The extended protein kinase C superfamily. Biochem J 332(Pt 2):281–292
11. Newton AC (2001) Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. Chem Rev 101:2353–2364
12. Nishizuka Y (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607–614
13. Ohno S, Nishizuka Y (2002) Protein kinase C isotypes and their specific functions: prologue. J Biochem 132:509–511
14. Besson A, Yong VW (2000) Involvement of p21(Waf1/Cip1) in protein kinase C alpha-induced cell cycle progression. Mol Cell Biol 20:4580–4590
15. Griner EM, Kazanietz MG (2007) Protein kinase C and other diacylglycerol effectors in cancer. Nat Rev Cancer 7:281–294
16. Gutcher I, Webb PR, Anderson NG (2003) The isoform-specific regulation of apoptosis by protein kinase C. Cell Mol Life Sci 60:1061–1070

17. Koivunen J, Aaltonen V, Peltonen J (2006) Protein kinase C (PKC) family in cancer progression. Cancer Lett 235:1–10
18. Mandil R, Ashkenazi E, Blass M et al (2001) Protein kinase Calpha and protein kinase Cdelta play opposite roles in the proliferation and apoptosis of glioma cells. Cancer Res 61:4612–4619
19. Ng T, Shima D, Squire A et al (1999) PKCalpha regulates beta1 integrin-dependent cell motility through association and control of integrin traffic. EMBO J 18:3909–3923
20. Nishizuka Y (1995) Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 9:484–496
21. Scaglione-Sewell B, Abraham C, Bissonnette M et al (1998) Decreased PKC-alpha expression increases cellular proliferation, decreases differentiation, and enhances the transformed phenotype of CaCo-2 cells. Cancer Res 58:1074–1081
22. Wetsel WC, Khan WA, Merchenthaler I et al (1992) Tissue and cellular distribution of the extended family of protein kinase C isoenzymes. J Cell Biol 117:121–133
23. Fournier DB, Chisamore M, Lurain JR et al (2001) Protein kinase C alpha expression is inversely related to ER status in endometrial carcinoma: possible role in AP-1-mediated proliferation of ER-negative endometrial cancer. Gynecol Oncol 81:366–372
24. Koren R, Ben Meir D, Langzam L et al (2004) Expression of protein kinase C isoenzymes in benign hyperplasia and carcinoma of prostate. Oncol Rep 11:321–326
25. Koren R, Langzam L, Paz A et al (2000) Protein kinase C (PKC) isoenzymes immunohistochemistry in lymph node revealing solution-fixed, paraffin-embedded bladder tumors. Appl Immunohistochem Mol Morphol 8:166–171
26. Langzam L, Koren R, Gal R et al (2001) Patterns of protein kinase C isoenzyme expression in transitional cell carcinoma of bladder. Relation to degree of malignancy. Am J Clin Pathol 116:377–385
27. Martinez-Gimeno C, Diaz-Meco MT, Dominguez I et al (1995) Alterations in levels of different protein kinase C isotypes and their influence on behavior of squamous cell carcinoma of the oral cavity: epsilon PKC, a novel prognostic factor for relapse and survival. Head Neck 17:516–525
28. Varga A, Czifra G, Tallai B et al (2004) Tumor grade-dependent alterations in the protein kinase C isoform pattern in urinary bladder carcinomas. Eur Urol 46:462–465
29. Ainsworth PD, Winstanley JH, Pearson JM et al (2004) Protein kinase C alpha expression in normal breast, ductal carcinoma in situ and invasive ductal carcinoma. Eur J Cancer 40:2269–2273
30. Gokmen-Polar Y, Murray NR, Velasco MA et al (2001) Elevated protein kinase C betaII is an early promotive event in colon carcinogenesis. Cancer Res 61:1375–1381
31. Kerfoot C, Huang W, Rotenberg SA (2004) Immunohistochemical analysis of advanced human breast carcinomas reveals down-regulation of protein kinase C alpha. J Histochem Cytochem 52:419–422
32. Neill GW, Ghali LR, Green JL et al (2003) Loss of protein kinase Calpha expression may enhance the tumorigenic potential of Gli1 in basal cell carcinoma. Cancer Res 63:4692–4697
33. Tsai JH, Hsieh YS, Kuo SJ et al (2000) Alteration in the expression of protein kinase C isoforms in human hepatocellular carcinoma. Cancer Lett 161:171–175
34. Edmondson HA, Steiner PE (1954) Primary carcinoma of the liver: a study of 100 cases among 48, 900 necropsies. Cancer 7:462–503
35. Yeh KT, Yang MY, Liu TC et al (2005) Abnormal expression of period 1 (PER1) in endometrial carcinoma. J Pathol 206:111–120
36. Yuo CY, Lin HH, Chang YS et al (2008) 5-(N-ethyl-N-isopropyl)-amiloride enhances SMN2 exon 7 inclusion and protein expression in spinal muscular atrophy cells. Ann Neurol 63:26–34
37. Engers R, Mrzyk S, Springer E et al (2000) Protein kinase C in human renal cell carcinomas: role in invasion and differential isoenzyme expression. Br J Cancer 82:1063–1069
38. Kamimura K, Hojo H, Abe M (2004) Characterization of expression of protein kinase C isozymes in human B-cell lymphoma: relationship between its expression and prognosis. Pathol Int 54:224–230
39. Weichert W, Gekeler V, Denkert C et al (2003) Protein kinase C isoform expression in ovarian carcinoma correlates with indicators of poor prognosis. Int J Oncol 23:633–639
40. Wu TT, Hsieh YH, Wu CC et al (2007) Overexpression of protein kinase C alpha mRNA in human hepatocellular carcinoma: a potential marker of disease prognosis. Clin Chim Acta 382:54–58
41. Chang KJ, Lin JK, Lee PH et al (1996) The altered activity of membrane-bound protein kinase C in human liver cancer. Cancer Lett 105:211–215
42. Hsieh YH, Wu TT, Tsai JH et al (2006) PKCalpha expression regulated by Elk-1 and MZF-1 in human HCC cells. Biochem Biophys Res Commun 339:217–225
43. Wu TT, Hsieh YH, Hsieh YS et al (2008) Reduction of PKC alpha decreases cell proliferation, migration, and invasion of human malignant hepatocellular carcinoma. J Cell Biochem 103:9–20
44. Heo KS, Kim DU, Kim L et al (2008) Activation of PKCbeta(II) and PKCtheta is essential for LDL-induced cell proliferation of human aortic smooth muscle cells via Gi-mediated Erk1/2 activation and Egr-1 upregulation. Biochem Biophys Res Commun 368:126–131
45. Nagahama K, Ogawa A, Shirane K et al (2008) Protein kinase C theta plays a fundamental role in different types of chronic colitis. Gastroenterol 134:459–469
46. Hayashi K, Altman A (2007) Protein kinase C theta (PKCtheta): a key player in T cell life and death. Pharmacol Res 55:537–544
47. Sims TN, Soos TJ, Xenias HS et al (2007) Opposing effects of PKCtheta and WASp on symmetry breaking and relocation of the immunological synapse. Cell 129:773–785
48. Ou WB, Zhu MJ, Demetri GD et al (2008) Protein kinase C-theta regulates KIT expression and proliferation in gastrointestinal stromal tumors. Oncogene 27:5624–5634
49. Belguise K, Sonenshein GE (2007) PKCtheta promotes c-Rel-driven mammary tumorigenesis in mice and humans by repressing estrogen receptor alpha synthesis. J Clin Invest 117:4009–4021
50. Cen L, Arnoczky KJ, Hsieh FC et al (2007) Phosphorylation profiles of protein kinases in alveolar and embryonal rhabdomyosarcoma. Mod Pathol 20:936–946
51. Despouy G, Joiner M, Le Toriellec E et al (2007) The TCL1 oncoprotein inhibits activation-induced cell death by impairing PKCtheta and ERK pathways. Blood 110:4406–4416
52. Kim KM, Kang DW, Moon WS et al (2006) PKCtheta expression in gastrointestinal stromal tumor. Mod Pathol 19:1480–1486
53. Gao Z, Wang Z, Zhang X et al (2007) Inactivation of PKCtheta leads to increased susceptibility to obesity and dietary insulin resistance in mice. Am J Physiol Endocrinol Metab 292:E84–91
54. Haasch D, Berg C, Clampit JE et al (2006) PKCtheta is a key player in the development of insulin resistance. Biochem Biophys Res Commun 343:361–368
55. Garaude J, Kaminski S, Charni S et al (2008) Impaired anti-leukemic immune response in PKCtheta-deficient mice. Mol Immunol 45:3463–3469
56. Schaffel R, Morais JC, Biasoli I et al (2007) PKC-beta II expression has prognostic impact in nodal diffuse large B-cell lymphoma. Mod Pathol 20:326–330
57. Bazarsky E, Wolfson M, Galron D et al (1997) Persistent measles virus infection of murine neuroblastoma cells differentially affects the expression of PKC individual isoenzymes. Virus Genes 15:227–234

58. Langzam L, Koren R, Gal R et al (2001) Patterns of protein kinase C isoenzyme expression in transitional cell carcinoma of bladder. Am J Clin Pathol 116:377–385
59. Filmus J, Capurro M (2004) Glypican-3 and alphafetoprotein as diagnostic tests for hepatocellular carcinoma. Mol Diagn 8:207–212
60. Lin YC, Lee PH, Yao YT et al (2007) Alpha-fetoprotein-producing pancreatic acinar cell carcinoma. J Formos Med Assoc 106:669–672
61. Yamada K, Fujioka Y, Ebihara Y et al (1994) Alpha-fetoprotein producing undifferentiated carcinoma of the bladder. J Urol 152:958–960
62. Chen DS, Sung JL, Sheu JC et al (1984) Serum alpha-fetoprotein in the early stage of human hepatocellular carcinoma. Gastroenterol 86:1404–1409
63. Sherman M (2001) Alphafetoprotein: an obituary. J Hepatol 34:603–605