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PKCα promotes generation of reactive oxygen species via DUOX2 in hepatocellular carcinoma

Jiajun Wang [a, 1], Miaomiao Shao [a, 1], Min Liu [a], Peike Peng [a], Lili Li [a], Weicheng Wu [a], Lan Wang [a], Fangfang Duan [b], Mingming Zhang [a], Shushu Song [a], Dongwei Jia [a, *], Yuanyuan Ruan [a, *], Jianxin Gu [a, b]

[a] Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Fudan University, 200032 Shanghai, PR China
[b] Institute of Biomedical Science, Fudan University, Shanghai, PR China

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ABSTRACT

Hepatocellular carcinoma (HCC) remains the second leading cause of cancer-related death worldwide, and elevated rates of reactive oxygen species (ROS) have long been considered as a hallmark of almost all types of cancer including HCC. Protein kinase C alpha (PKCα), a serine/threonine kinase among conventional PKC family, is recognized as a major player in signal transduction and tumor progression. Overexpression of PKCα is commonly observed in human HCC and associated with its poor prognosis. However, how PKCα is involved in hepatocellular carcinogenesis remains not fully understood. In this study, we found that among the members of conventional PKC family, PKCα, but not PKCβI or βII, promoted ROS production in HCC cells. PKCα stimulated generation of ROS by up-regulating DUOX2 at post-transcriptional level. Depletion of DUOX2 abrogated PKCα-induced activation of AKT/MAPK pathways as well as cell proliferation, migration and invasion in HCC cells. Moreover, the expression of DUOX2 and PKCα was well positively correlated in both HCC cell lines and patient samples. Collectively, our findings demonstrate that PKCα plays a critical role in HCC development by inducing DUOX2 expression and ROS generation, and propose a strategy to target PKCα/DUOX2 as a potential adjuvant therapy for HCC treatment.

1. Introduction

Liver cancer is among the most common and lethal cancers in the human population, ranked the sixth-most frequent neoplasm and the second-most common cause of cancer-related death worldwide [1]. Among primary liver cancers, hepatocellular carcinoma (HCC) accounts for 70%–90% of the total liver cancer burden worldwide [2]. Though surveillance can lead to early diagnosis when the tumor might be resectable, HCC is generally diagnosed at an advanced stage and has a poor prognosis, owing to the underlying liver disease and the lack of effective therapeutic options [3,4]. Therefore, revealing the mechanisms of tumor development and occurrence is critical for improving the treatment of HCC in clinical practice.

Protein kinase C (PKC) is a multigene family that encodes at least 11 distinct isoforms of lipid-regulated serine/threonine kinases, which can catalyze numerous biochemical reactions critical to the function of many cellular constituents [5]. Based on the structural and activational characteristics, PKC genes can be classified into 3 groups: conventional PKCs, novel PKCs, and atypical PKCs [6]. PKCα, a member of conventional PKC, is ubiquitously expressed in all tissues of human beings, and plays important roles in the control of major cellular functions, including proliferation, apoptosis, differentiation, motility and so on [7]. Though mutations in PKC isozymes are rare occurrences in tumor genetics, PKCα is implicated in malignant transformation through enhancing multiple cellular signaling pathways, and overexpression of PKCα has been described in several kinds of human malignancies, including HCC [8–11].

Reactive oxygen species (ROS) are a diverse class of radical species that are produced in all cells as a normal byproduct of metabolic processes, and function as key secondary messengers in numerous signaling pathways [12]. In highly proliferative cancer

* Corresponding authors.
E-mail addresses: jiadongwei@fudan.edu.cn (D. Jia), yuanyuanruan@fudan.edu.cn (Y. Ruan).
[1] These authors contributed equally to this work.

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cells, ROS regulation is crucial owing to the presence of oncogenic mutations that promote aberrant metabolism and protein translation, resulting in increased rates of ROS production [13]. Elevated ROS level commonly causes genomic instability and thereby promotes tumorigenesis [14]. It has been recognized that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOXs) is an important enzymatic source for the production of ROS depending on the types of cells [15]. The NOX family enzymes plays critical roles in host defense, posttranslational processing of proteins, cellular signaling, regulation of gene expression and cell differentiation [16]. Increased NOX activity also contributes to a large number of pathologies, including cardiovascular diseases, neurodegeneration and cancers [16].

Though the tumorigenic role of PKCα has been explored, how this serine/threonine kinase is involved in tumor promotion remains only partially understood. In this study, we found that the expression of DUOX2, a member of NOX family, was induced by PKCα in HCC cells. DUOX2 was required for PKCα-mediated ROS production, AKT/MAPK signaling activation and tumor progression. Our data suggest that aberrant activation of PKCα/DUOX2 pathway may play a critical role in hepatocellular carcinogenesis.

2. Materials and methods

2.1. Patients and tumor samples

Tumor tissues of 40 primary HCC cases, surgically resected at Huashan Hospital of Fudan University (Shanghai, China) from 2006 to 2008, were fixed in 10% formalin and embedded in paraffin. None of the patients received clinical treatment before surgery. This study was approved by the Ethics Committee of Fudan University. All donors were informed of the aim of the study and gave consent to donate their samples.

2.2. Antibodies and reagents

Rabbit anti-ERK, -phospho-ERK, -AKT, -phospho-AKT, -phospho-p38, -p38, and -β-actin antibodies were purchased from Cell Signaling Technology. Rabbit anti-NOX3, -NOX5 antibodies and mouse anti-PKCα, -DUOX1, -DUOX2 antibodies were obtained from Santa Cruz Biotechnology. Rabbit anti-NOX2 and -NOX4 antibodies were purchased from Proteintech. Go6976 was obtained from BioVision. Rabbit anti-NOX1 antibody was purchased from Abcam. LY333531, Dihydroethidium (DHE), Rhodamine 123 and N-acetylcysteine (NAC) were from Sigma Aldrich.

2.3. Cell lines

All cell lines were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), and cultured in Dulbecco's Modified Eagle's Medium (Sigma Aldrich, USA) supplemented with 10% fetal bovine serum (Gibco) at 37 °C in a humidified atmosphere with 5% CO_2.

2.4. Plasmid, RNA interference and transfection

pENTER/PKCα construct was purchased from Vigene Bioscience. The control siRNA (UUCUCCGAACGUGUCACGUTT) and PKCα siRNA (CCAUCGGAUUGUUCUUUCUUCAUAA) were designed according to a previous report [17]. DUOX2 siRNA was purchased from Biotend. All transfections were performed using Lipofectamine2000 (Invitrogen) according to manufacturer's instructions.

2.5. Western-blot

Western-blot analysis was carried out according to our previous report [18].

2.6. Real-time PCR

Briefly, total RNA was extracted with TRIZOL (Invitrogen) according to manufacturer's instructions. Quality of the total RNA was detected by spectrophotometer (Pharmacia Biotech RNA/DNA Calculator). About 3 μg total RNA from each sample was used to perform reverse-transcribed by using RNA PCR Kit AMV (Takara). Real-time PCR was performed using SYBR Green Premix Ex Taq Ver. 3.0 (Takara) and detected by StepOne plus (ABI). The following primers were used: *gapdh* forward: 5′-GAG CGA GAC CCC ACT AAC AT-3′; *gapdh* reverse: 5′-TCT CCA TGG TGG TGA AGA CA-3′; *PRKCA* forward: 5′-ATG GAT CAC ACT GAG AAG AGG-3′; *PRKCA* reverse: 5′-AAG GTT GTT GGA AGG TTG TTT-3′; *DUOX2* forward: 5′-ACG CAG CTC TGT GTC AAA GGT-3′; *DUOX2* reverse: 5′-TGA TGA ACG AGA CTC GAC CAG GC-3′.

2.7. ROS detection

Briefly, HCC cells were incubated with DHE (1 μM) or Rhodaming 123 (1 μM), harvested by trypsinization, washed with PBS and detected by flow cytometry. Mean fluorescence intensity (MFI) was calculated after correction for autofluorescence.

2.8. Cell proliferation assay

Cell proliferation was determined using Cell Counting Kit-8 (CCK-8) (Beyotime Inst. Biotech, China). Briefly, cells were transfected as indicated, seeded in a 96-well plate, and incubated with WST-8 dye at 37 °C for 0.5 h. Cell viability was determined by the absorbance at 450 nm using a Universal Microplate Reader (Bio-Tek Instrument Inc.). All assays were performed in triplicate.

2.9. Transwell assay

The migratory and invasive abilities of Huh7 cells were assayed in BD Falcon 24-well plates with transwell inserts containing 8 μm pore filters (Millipore, MA, USA), and the invasive activity of Huh7 cells was tested with BD Matrigel coated filters. Briefly, Huh7 cells were transfected as indicated. Then cells were resuspended and seeded into the upper chamber of the assay system. The infiltrated cells were stained with crystal violet, and cell numbers were counted from five visions. Each experiment was repeated at 3 times.

2.10. Immunohistochemistry

Briefly, tissue sections on glass slides were deparaffinized, dehydrated and subjected to antigen retrieval. Then sections were blocked by UltraVision Protein Block (Thermo Scientific) followed by primary antibodies incubation. After DAB staining, slides were counterstained with hematoxylin, mounted and captured by Nikon microscope. Immunohistochemical scoring was determined according to our previous report [18].

2.11. Statistical analysis

All results are presented as the means ± S.D. Differences between groups were calculated using two-tailed Student's t-test. Correlation between PKCα and DUOX2 expression was determined

by Pearson's $\chi 2$ test. Statistical significance was determined at the level of $P < 0.05$.

3. Results

3.1. PKCα induces ROS production in hepatocellular carcinoma

Elevated ROS level, which is commonly detected in almost all types of cancer, plays a critical role in inducing genomic instability and tumorigenesis [19]. To explore whether PKCα was involved in ROS generation in HCC, we first examined the effect of Go6976, a PKCα/βI inhibitor, on the production of ROS in HCC cell lines. As shown in Fig. 1A and B, treatment of Go6976 suppressed the level of ROS in dose-dependent manner in both SK-Hep1 and Huh7 cells. However, administration of LY333531, a potent inhibitor of PKCβI/βII, showed little effect on DHE staining in these two cell lines.

To better understand the role of PKCα in ROS production in HCC, we also examined whether the level of ROS changed in response to altered PKCα expression. As shown in Fig. 1C, overexpression of PKCα promoted ROS production in Huh7 cells by DHE and Rhodamine 123 staining. In addition, knock-down of PKCα using specific siRNA remarkably inhibited ROS accumulation in SK-Hep1 cells. These results suggest that among the members of conventional PKCs, PKCα, but not PKCβI or PKCβII, was involved in the production of ROS in hepatocellular carcinoma.

3.2. PKCα induces ROS production by regulating DUOX2 expression

It has been reported that NOXs family plays an important role in ROS generation in human HCC cells [20]. To understand how PKCα modulated the level of ROS in HCC cells, we next examined the effect of PKCα on the expression of NOXs family. As shown in Fig. 2A and B, overexpression of PKCα promotes the expression of DUOX2 in Huh7 cells, while depletion of PKCα using specific siRNA reduced DUXO2 protein level in SK-Hep1 cells. Either overexpression or depletion of PKCα showed little effect on the expression of other members of NOXs family (Fig. 2A and B). We did not detect endogenous DUOX1 expression in Huh7 and SK-Hep1 cells, probably due to that DUOX1 was commonly epigenetically silenced by promoter hypermethylation in human HCC [21]. In addition, we also found that overexpression or depletion of PKCα showed little effect on DUOX2 mRNA levels in HCC cells, suggesting that PKCα modulated DUOX2 expression at post-transcriptional level (Fig. 2C and D).

We next determined whether DUOX2 was involved in PKCα-mediated ROS production in HCC. As shown in Fig. 2E, depletion of

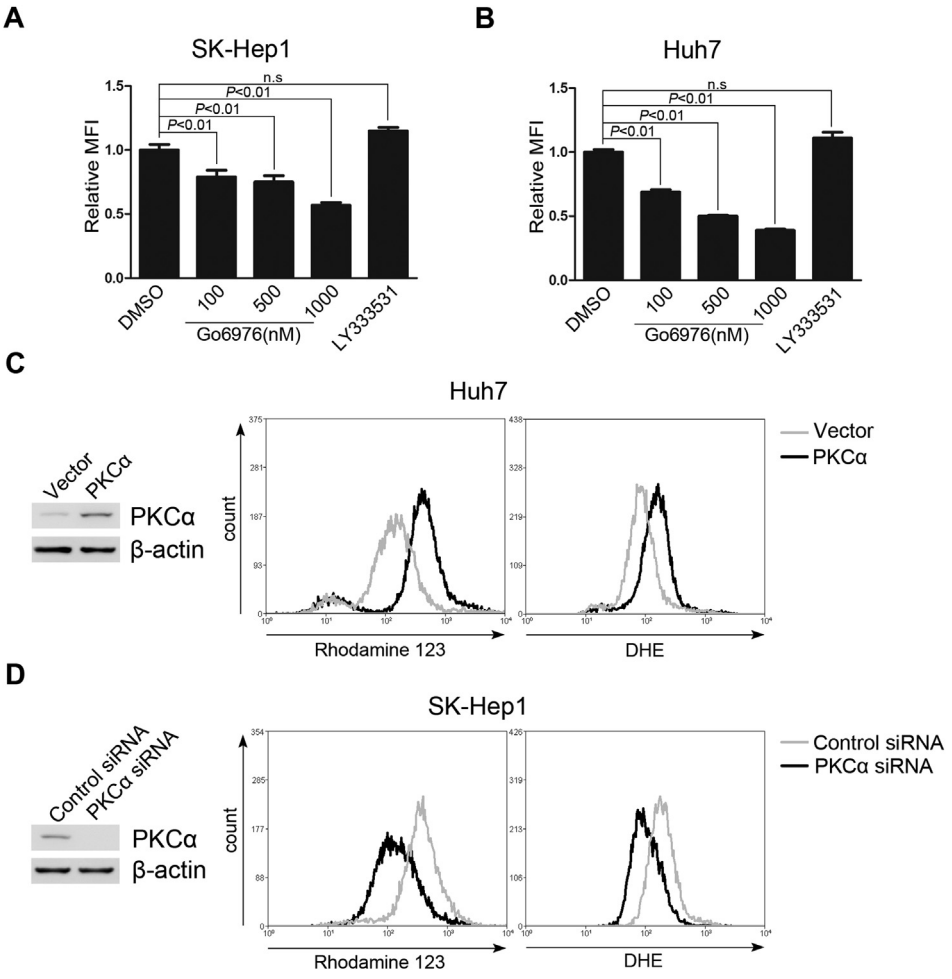

Fig. 1. PKCα promotes the generation of ROS in hepatocellular carcinoma. (A–B) SK-Hep1 (A) and Huh7 (B) cells were treated with Go6976 (100 nM, 500 nM, 1000 nM) or LY333531 (200 nM), incubated with DHE and applied to flow cytometry analysis. (C) Huh7 cells were transiently transfected with empty vector or PKCα. 48 h later, cells were incubated with DHE or Rhodamine 123, and subjected to flow cytometry analysis. (D) SK-Hep1 cells were transiently transfected with control or PKCα siRNA. 72 h later, cells were incubated with DHE or Rhodamine 123, and subjected to flow cytometry analysis. n.s., no significance.

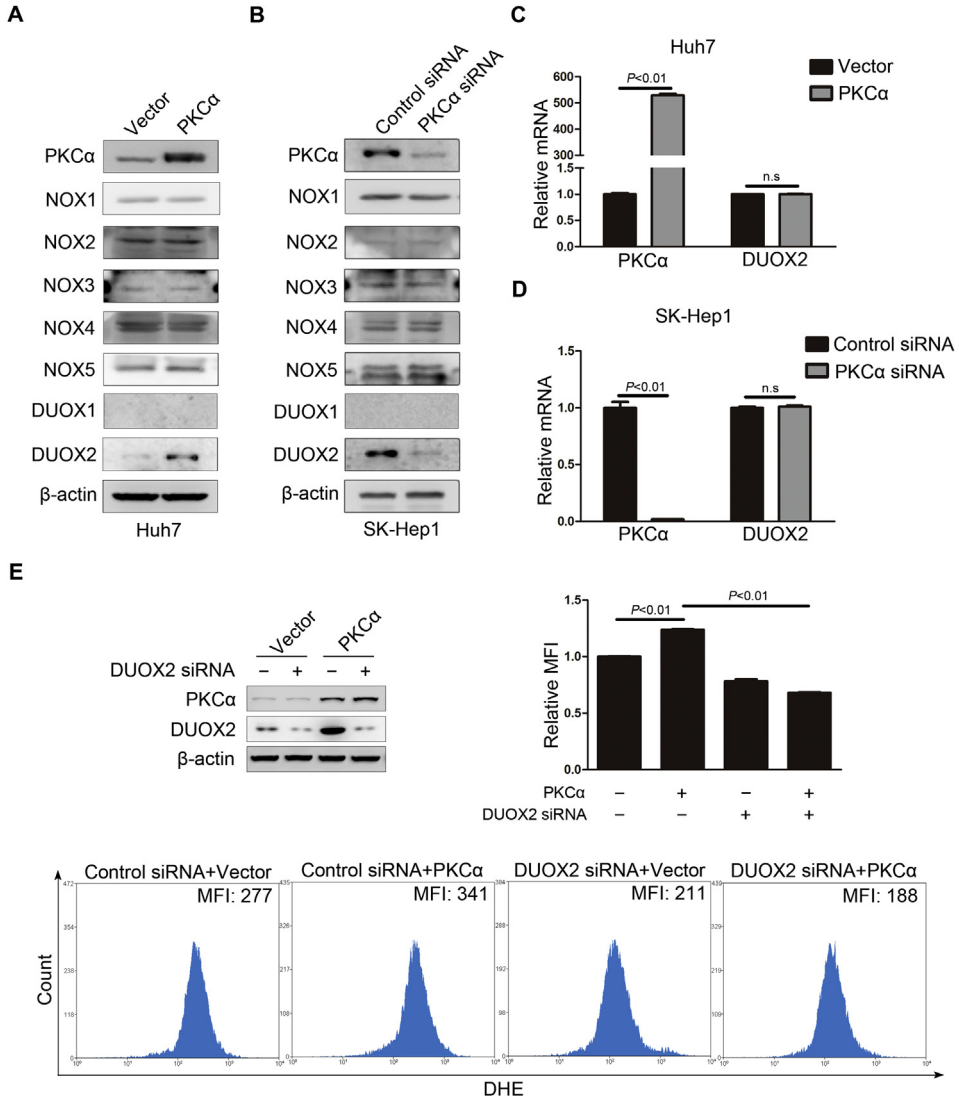

Fig. 2. PKCα induces ROS production by regulating DUOX2 expression. (A) Huh7 cells were transfected with constructs as indicated, and applied to western blot analysis. (B) SK-Hep1 cells were transfected with siRNA as indicated, and applied to western blot analysis. (C) Huh7 cells were transfected in (A), and applied to real-time PCR analysis. (D) SK-Hep1 cells were transfected as in (B), and applied to real-time PCR analysis. (E) Huh7 cells were transfected with vector or PKCα construct, along with control or DUOX2 siRNA. The level of intracellular ROS was measured by DHE staining and flow cytometry analysis. Images were representative of three independent experiments. n.s., no significance.

DUOX2 attenuated basal level of ROS, and also dramatically blocked PKCα-induced ROS accumulation in Huh7 cells. These results suggest that PKCα stimulates ROS production by up-regulating DUOX2 in hepatocellular carcinoma.

3.3. PKCα promotes AKT/MAPK activation as well as cell proliferation, migration and invasion via DUOX2 in hepatocellular carcinoma

ROS are involved as key secondary messengers in numerous signaling pathways. We next evaluated whether DUOX2-mediated ROS accumulation was involved in the oncogenic effect of PKCα in HCC. As shown in Fig. 3A, overexpression of PKCα enhanced the phosphorylation of AKT, p38 and ERK in Huh7 cells, and depletion of DUOX2 or administration of antioxidant NAC suppressed PKCα-induced activation of AKT, p38 and ERK. We also determined whether DUOX2 was involved in PKCα-mediated proliferation, migration and invasion in HCC. As shown in Fig. 3B, CCK8 assay revealed that overexpression of PKCα increased the viability of Huh7 cells, and knock-down of DUOX2 using specific siRNA abolished the pro-proliferative effect of PKCα in Huh7 cells. Moreover, transwell analysis also revealed that depletion of DUOX2 blocked PKCα-stimulated migration and invasion of Huh7 cells (Fig. 3C and D). These results imply that DUOX2 is required for the oncogenic effect of PKCα in hepatocellular carcinoma.

3.4. The expression of DUOX2 is positively correlated with PKCα in hepatocellular carcinoma

Since up-regulation of DUOX2 was critical for the oncogenic role of PKCα in HCC, we next determined whether expression of DUOX2 was correlated with PKCα in HCC. As shown in Fig. 4A and B, western blot analysis revealed that the protein levels of DUOX2 and PKCα exhibited similar pattern in immortalized L02 hepatic cell and different kinds of HCC cell lines, and statistical analysis confirmed the correlated expression of DUOX2 and PKCα in these cell lines ($R^2 = 0.763$, $P = 0.023$). Immunohistochemistry assay also revealed that the protein expression of DUOX2 and PKCα was well positively

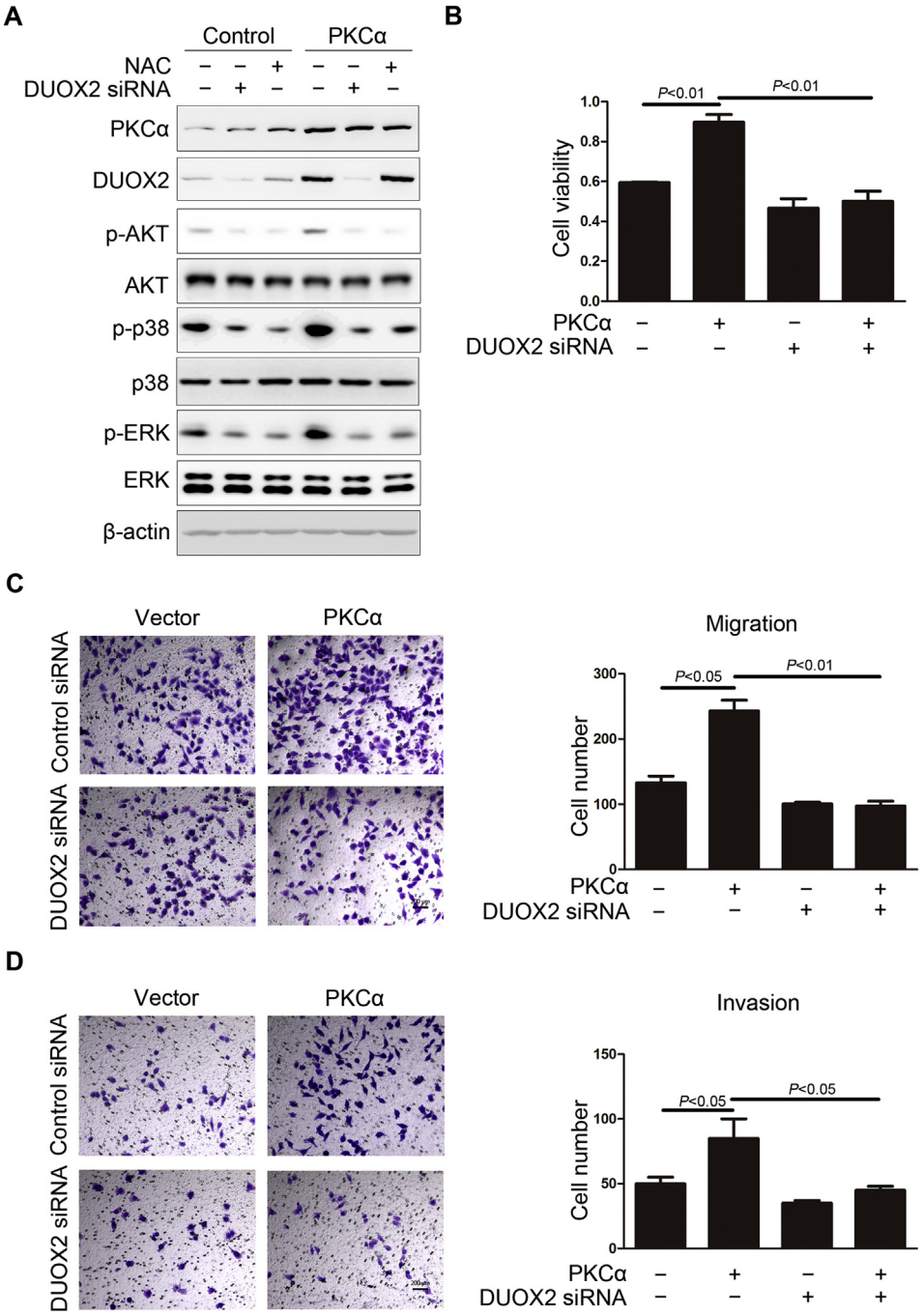

Fig. 3. PKCα promotes AKT/MAPK activation as well as cell proliferation, migration and invasion via DUOX2. (A) Huh7 cells were transfected with constructs or siRNA as indicated, along with or without treatment with NAC (5 mM). Then cell lysates were applied to western blot analysis. (B–D) Huh7 cells were transfected as indicated, followed by CCK8 analysis (B), migration assay (C) and invasion assay (D). In (C) and (D), images were representative of three independent experiments, and scale bar = 200 μm.

correlated in 40 cases of HCC samples ($R^2 = 0.889$, $P < 0.001$) (Fig. 4C and D). These results suggest that the expression of DUOX2 is positively correlated with PKCα in hepatocellular carcinoma.

4. Discussion

Hepatic carcinogenesis is thought to involve ROS-induced DNA damage and/or mitogenic signaling. ROS plays a critical role in development of HCC in a variety of rodent models including mice overexpressing c-myc and TGF-α and exposure to carcinogens such as peroxisome proliferator compounds and diethylnitrosamine [22]. Therefore, exploring the underlying mechanism of ROS generation would help in understanding hepatocellular carcinogenesis and providing new therapeutic targets. PKC was originally recognized as a major player in cellular signal transduction, and accumulating evidence have confirmed the involvement of PKC isozymes in mitogenesis, survival and malignant transformation through their increased or decreased participation in various cellular signaling pathways [23]. In this study, our data demonstrate that PKCα, but not PKCβI or PKCβII, promoted ROS production in HCC cells. Further research revealed that increased expression of DUOX2 was involved in PKCα-induced ROS generation and HCC

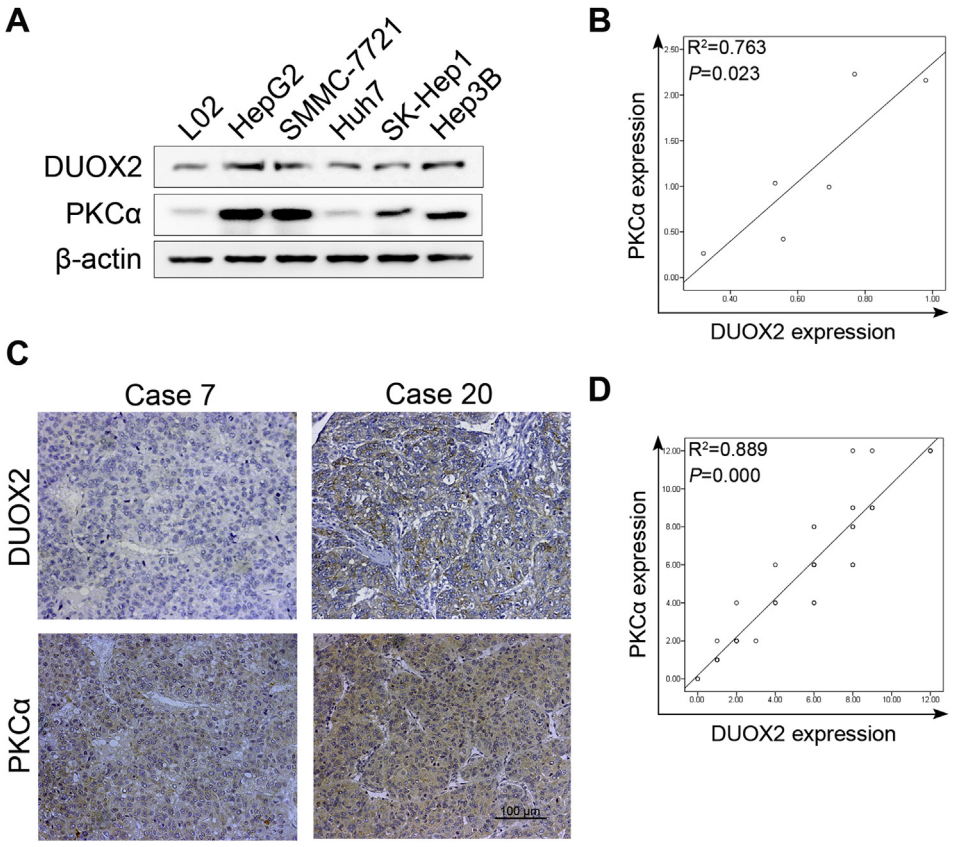

Fig. 4. Correlated expression of PKCα and DUOX2 in hepatocellular carcinoma. (A–B) Endogenous expression of DUOX2 and PKCα in immortalized L02 hepatocyte and various kinds of HCC cell lines was examined by western blot (A), and the correlation between DUOX2 and PKCα expression was determined by Pearson's $\chi 2$ test (B). (C–D) Endogenous expression of DUOX2 and PKCα in HCC samples was examined by immunohistochemical staining (n = 40), and the correlation between DUOX2 and PKCα staining was determined by Pearson's $\chi 2$ test (D). In (C), representative images of DUOX2 and PKCα staining in two cases were shown, and scale bar = 100 μm.

progression *in vitro*. Our data imply that the novel PKCα/DUOX2/ROS pathway may participate in the development of HCC, and provide evidence for better understanding the mechanism of ROS production during hepatic carcinogenesis.

NOXs family is considered as a major source of non-mitochondrial ROS, and the correlation between distinct PKC isozymes and NOXs family has been reported. In vascular smooth muscle cells, gene silencing of PKCδ by RNA interference significantly suppressed the PGF2α-induced increase in NOX1 mRNA and protein [24]. In mouse mesangial cells, the increased activity of PKCε under high glucose condition decreased the expression of NOX4 through MAPK pathway [25]. In addition to modulating ROS production through NOXs, PKC could also regulate the generation of mitochondrial ROS. It has been reported that the PKCβ isozyme is responsible for the activation/phosphorylation of the mitochondrial $p66^{shc}$ protein, which can bind to cytochrome c and stimulate the generation of ROS [26]. In our study, we found that PKCα promoted the production of ROS by stimulating DUOX2 expression in HCC, suggesting that the mechanisms for PKCs-mediated ROS generation may vary depending on the types of cells and PKC isoforms. Interestingly, a previous study also demonstrated that PKCα could be activated by TPA-triggered ROS generation in HCC [27]. Therefore, PKCα and ROS may form a positive feedback loop to induce the carcinogenesis and development of HCC.

In this study, we found that PKCα promoted DUOX2 protein level without affecting its mRNA expression, suggesting that PKCα regulated DUOX2 expression at post-transcriptional level (Fig. 2). So far, how the members of NOXs family are post-transcriptionally regulated remains little understood. A previous study demonstrated that PKC inhibitor Ro318220 specifically inhibited PMA-stimulated DUOX2 phosphorylation and H_2O_2 production in COS-7 cells, suggesting PKC is a critical regulator of DUOX2 phosphorylation [28]. Moreover, it also has been reported that PKCα can phosphorylate NOX5 and enhance its activity [29]. Therefore, it is possible that PKCα may promote DUOX2 expression and activity by stimulating its phosphorylation. And this assumption needs further investigation.

PKCα is ubiquitously expressed in many tissues and associated with cell proliferation, apoptosis and cell motility. PKCα has been recognized as a potent oncogene in several kinds of human malignancies, including glioblastoma, pancreatic cancer, breast cancer, and prostate cancer [30]. In HCC, it is reported that high expression of PKCα was positively correlated with tumor size and TNM stage, and also predicted poor prognosis [11]. Reduction of PKCα decreased cell proliferation, migration, and invasion of human HCC cells [31]. However, contradictory results challenging the tumorigenic effect of PKCα have also been described depending on the tumor type [32]. Moreover, a recent report also suggested that PKCα generally functioned as a tumor suppressor, and mutation of PKCα exerted bioplastic and metastasizing effects in cancer cells [33]. Therefore, the exact role of PKCα in carcinogenesis needs to be further elucidated. In our study, we found that up-regulation of DUOX2 was required for the oncogenic effect of PKCα in HCC cells. Depletion of DUOX2 suppressed PKCα-induced AKT/MAPK activation as well as cell proliferation, migration and invasion (Fig. 3). Our data provide novel evidence for better understanding the role of PKCα in hepatocellular carcinogenesis.

Currently, most patients are diagnosed with HCC at advanced stage, and there is no standard treatment for unresectable HCC.

Transarterial chemoembolization, which is widely used in non-surgical cases, is only effective in patients with preserved liver function and absence of extrahepatic spread [34,35]. In recent years, sorafenib, a multikinase inhibitor that has been used in patients with advanced HCC, only increases the median overall survival from 7.9 to 10.7 months [36]. Therefore, it is of great potential in developing new agents and strategies for this group of patients. Indeed, several inhibitors of PKC and NOX family have been designed and developed, and application of these inhibitors into clinical practice is also being explored [32,37]. Our data suggest that DUOX2 plays a critical role in PKCα-mediated ROS production and tumor progression in HCC, and propose a strategy to target PKCα/DUOX2 as a potential adjuvant therapy in combination with other methods for HCC treatment.

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References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global Cancer Statistics, 2012, CA Cancer J. Clin. 65 (2015) 87–108.
[2] D. Schottenfeld, J.F. Fraumeni, Cancer Epidemiology and Prevention, third ed., Oxford University Press, Oxford ; New York, 2006.
[3] J.M. Llovet, A. Burroughs, J. Bruix, Hepatocellular carcinoma, Lancet 362 (2003) 1907–1917.
[4] J. Bruix, M. Sherman, J.M. Llovet, M. Beaugrand, R. Lencioni, A.K. Burroughs, E. Christensen, L. Pagliaro, M. Colombo, J. Rodes, E.P.E. HCC, Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL Conference, J. Hepatol. 35 (2001) 421–430.
[5] Y. Nishizuka, Protein kinases .5. Protein-Kinase-C and lipid signaling for sustained cellular-responses, Faseb J. 9 (1995) 484–496.
[6] E.M. Griner, M.G. Kazanietz, Protein kinase C and other diacylglycerol effectors in cancer, Nat. Rev. Cancer 7 (2007) 281–294.
[7] S. Nakashima, Protein kinase C alpha (PKC alpha): regulation and biological function, J. Biochem. 132 (2002) 669–675.
[8] C.A. Obrian, V.G. Vogel, S.E. Singletary, N.E. Ward, Elevated protein kinase-C expression in human-breast tumor-biopsies relative to normal breast-tissue, Cancer Res. 49 (1989) 3215–3217.
[9] G.P. Perletti, C. Smeraldi, D. Porro, F. Piccinini, Involvement of the alpha-isoenzyme of protein-kinase-C in the growth-inhibition induced by phorbol esters in Mh1c1 hepatoma-cells, Biochem. Biophys. Res. Commun. 205 (1994) 1589–1594.
[10] P. Henttu, P. Vihko, The protein kinase C activator, phorbol ester, elicits disparate functional responses in androgen-sensitive and androgen-independent human prostatic cancer cells, Biochem. Biophys. Res. Commun. 244 (1998) 167–171.
[11] T.T. Wu, Y.H. Hsieh, C.C. Wu, Y.S. Hsieh, C.Y. Huang, J.Y. Liu, Overexpression of protein kinase C alpha mRNA in human hepatocellular carcinoma: a potential marker of disease prognosis, Clin. Chim. Acta 382 (2007) 54–58.
[12] V.J. Thannickal, B.L. Fanburg, Reactive oxygen species in cell signaling, Am. J. Physiol. Lung Cell. Mol. Physiol. 279 (2000) L1005–L1028.
[13] R.A. Cairns, I.S. Harris, T.W. Mak, Regulation of cancer cell metabolism, Nat. Rev. Cancer 11 (2011) 85–95.
[14] M. Schieber, N.S. Chandel, ROS function in redox signaling and oxidative stress, Curr. Biol. 24 (2014) R453–R462.
[15] J.D. Lambeth, NOX enzymes and the biology of reactive oxygen, Nat. Rev. Immunol. 4 (2004) 181–189.
[16] K. Bedard, K.H. Krause, The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology, Physiol. Rev. 87 (2007) 245–313.
[17] G.K. Lonne, L. Cornmark, I.O. Zahirovic, G. Landberg, K. Jirstrom, C. Larsson, PKC alpha expression is a marker for breast cancer aggressiveness, Mol. Cancer 9 (2010).
[18] Y.Y. Ruan, L.L. Sun, Y.Q. Hao, L.J. Wang, J.J. Xu, W. Zhang, J.H. Xie, L. Guo, L. Zhou, X.J. Yun, H.G. Zhu, A.G. Shen, J.X. Gu, Ribosomal RACK1 promotes chemoresistance and growth in human hepatocellular carcinoma, J. Clin. Invest. 122 (2012) 2554–2566.
[19] G.Y. Liou, P. Storz, Reactive oxygen species in cancer, Free Radic. Res. 44 (2010) 479–496.
[20] R. Coriat, C. Nicco, C. Chereau, O. Mir, J. Alexandre, S. Ropert, B. Weill, S. Chaussade, F. Goldwasser, F. Batteux, Sorafenib-induced hepatocellular carcinoma cell death depends on reactive oxygen species production in vitro and in vivo, Mol. Cancer Ther. 11 (2012) 2284–2293.
[21] Q.X. Ling, W. Shi, C. Huang, J.M. Zheng, Q. Cheng, K.K. Yu, S.S. Chen, H. Zhang, N. Li, M.Q. Chen, Epigenetic silencing of dual oxidase 1 by promoter hypermethylation in human hepatocellular carcinoma, Am. J. Cancer Res. 4 (2014) 508–517.
[22] S. De Minicis, T. Kisseleva, H. Francis, G.S. Baroni, A. Benedetti, D. Brenner, D. Alvaro, G. Alpini, M. Marzioni, Liver carcinogenesis: rodent models of hepatocarcinoma and cholangiocarcinoma, Dig. Liver Dis. 45 (2013) 450–459.
[23] J.-H. Kang, Protein kinase C (PKC) isozymes and cancer, New J. Sci. 2014 (2014) 1–36.
[24] C.Y. Fan, M. Katsuyama, C. Yabe-Nishimura, PKC delta mediates up-regulation of NOX1, a catalytic subunit of NADPH oxidase, via transactivation of the EGF receptor: possible involvement of PKC delta in vascular hypertrophy, Biochem. J. 390 (2005) 761–767.
[25] N. Osawa, T. Hayashi, T. Uzu, T. Babazono, S. Babazono, Y. Iwamoto, A. Kashiwagi, S. Maeda, High glucose-induced activation of PKC epsilon decreases the expression of bone morphogenetic protein 4 (Bmp4) and NADPH oxidase 4 (Nox4) through MAPK-dependent pathway in cultured mouse mesangial cells, Diabetes 56 (2007). A200–A200.
[26] E. Migliaccio, M. Giorgio, S. Mele, G. Pelicci, P. Reboldi, P.P. Pandolfi, L. Lanfrancone, P.G. Pelicci, The p66shc adaptor protein controls oxidative stress response and life span in mammals, Nature 402 (1999) 309–313.
[27] W.S. Wu, R.K. Tsai, C.H. Chang, S. Wang, J.R. Wu, Y.X. Chang, Reactive oxygen species mediated sustained activation of protein kinase C alpha and extracellular signal-regulated kinase for migration of human hepatoma cell Hepg2, Mol. Cancer Res. 4 (2006) 747–758.
[28] S. Rigutto, C. Hoste, H. Grasberger, M. Milenkovic, D. Communi, J.E. Dumont, B. Corvilain, F. Miot, X. De Deken, Activation of Dual Oxidases Duox1 and Duox2 differential regulation mediated by camp-dependent protein kinase and protein kinase c-dependent phosphorylation, J. Biol. Chem. 284 (2009) 6725–6734.
[29] F. Chen, Y. Yu, S. Haigh, J. Johnson, R. Lucas, D.W. Stepp, D.J. Fulton, Regulation of NADPH oxidase 5 by protein kinase C isoforms, PLoS One 9 (2014) e88405.
[30] A.R. Hanauske, K. Sundell, M. Lahn, The role of protein kinase C-alpha (PKC-alpha) in cancer and its modulation by the novel PWC-alpha-specific inhibitor aprinocarsen, Curr. Pharm. Des. 10 (2004) 1923–1936.
[31] T.T. Wu, Y.H. Hsieh, Y.S. Hsieh, J.Y. Liu, Reduction of PKC alpha decreases cell proliferation, migration, and invasion of human malignant hepatocellular carcinoma, J. Cell. Biochem. 103 (2008) 9–20.
[32] G. Martiny-Baron, D. Fabbro, Classical PKC isoforms in cancer, Pharmacol. Res. 55 (2007) 477–486.
[33] C.E. Antal, A.M. Hudson, E. Kang, C. Zanca, C. Wirth, N.L. Stephenson, E.W. Trotter, L.L. Gallegos, C.J. Miller, F.B. Furnari, T. Hunter, J. Brognard, A.C. Newton, Cancer-associated protein kinase C mutations reveal kinase's role as tumor suppressor, Cell 160 (2015) 489–502.
[34] J. Bruix, L. Boix, M. Sala, J.M. Llovet, Focus on hepatocellular carcinoma, Cancer Cell 5 (2004) 215–219.
[35] A. Forner, M.I. Real, M. Varela, J. Bruix, Transarterial chemoembolization for patients with hepatocellular carcinoma, Hepatol. Res. 37 (2007) S230–S237.
[36] J.M. Llovet, S. Ricci, V. Mazzaferro, P. Hilgard, E. Gane, J.F. Blanc, A.C. de Oliveira, A. Santoro, J.L. Raoul, A. Forner, M. Schwartz, C. Porta, S. Zeuzem, L. Bolondi, T.F. Greten, P.R. Galle, J.F. Seitz, I. Borbath, D. Haussinger, T. Giannaris, M. Shan, M. Moscovici, D. Voliotis, J. Bruix, S.I.S. Grp, Sorafenib in advanced hepatocellular carcinoma, N. Engl. J. Med. 359 (2008) 378–390.
[37] J.A. Kim, G.P. Neupane, E.S. Lee, B.S. Jeong, B.C. Park, P. Thapa, NADPH oxidase inhibitors: a patent review, Expert Opin. Ther. Pat. 21 (2011) 1147–1158.