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Review

Protein Kinase C Alpha Expression in Human Hepatocellular Carcinoma

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Protein kinase C (PKC), which contains ten isozymes with distinct enzymological characteristics and intracellular localization, has been believed to be correlated with tumor proliferation, migration and invasion. A recent study found that the PKCα was significantly expressed in the human hepatocellular carcinoma (HCC), and positively correlated with tumor size, tumor stage and mortality rate. It was also found that PKCα played a critical role in cell proliferation, migration and invasion of the poorly differentiated human hepatoma cell lines (HA22T/VGH and SK-Hep-1). In this review, the PKCα signaling in both down-stream and up-stream pathways in HCC cells was discussed.

Key Words: protein kinase Cα, human hepatocellular carcinoma

Introduction

Protein kinase C (PKC), discovered by Nishizuka *et al*., is a kind of Ser/Thr protein kinase, which contains at least ten isoenzymes (24). They are divided into 3 groups of PKC isoenzymes: conventional, novel, and atypical. The conventional PKC isoenzymes consist of α, βI, βII, γ. The novel group consists of δ, ε, γ, μ, and the atypical group consists of ξ, ι. PKC is known to be involved in tumor promotion and progression (22), and high levels of PKC expression can be found in breast, prostate, urinary bladder, and lung cancers (15-17, 23, 32). PKC has also been known to become malignant through transfection. For example, when PKC β, ε, or γ is introduced into a cell, it induces fibroblast transformation (2, 3), or, when PKCα is introduced into an MCF-7 breast cancer cell, it promotes cell migration and invasion (36). Some experiments tried to decrease the PKCα expression through gene knockdown, such as antisense PKCα treatment on human lung carcinoma cells, human gastric cancer cells, human U87 glioma cell line, and human colon carcinoma cells, to inhibit cell growth (12, 20, 21, 35). Another experiment used a PKCα/β inhibitor Go6976 on urinary bladder cancer cells to inhibit cell invasion (15). Positive results from these experiments suggested that PKCα is a practicable research direction in understanding cancer development.

Hepatocellular carcinoma (HCC) is a worldwide cancer studied by many scientists (29). HCC can be caused by infectious agents (hepatitis B virus, hepatitis C virus, *etc*.), pathology (chronic liver disease, neonatal hepatitis, *etc*.), diet (aflatoxin consumption, dietary iron overload, *etc*.), hormone imbalance (oral contraceptives, anabolic steroids, *etc*.), pollutants (vinyl chloride, cigarette smoking, *etc*.), genetic inheritance (hereditary tyrosinemia, a1-antitrypsin deficiency, *etc*.), and other factors (elevated TGF-α, age, gender, *etc*.) (7). There is an ongoing research into HCC treatment, especially in Taiwan, where HCC is common.

Investigators used many indicators to detect HCC in patients, but few of them can determine the type of cancer specifically. Therefore, the researchers continue to find other indicators which react to very specific cancer types and develop more accurate anticancer treatment. Many papers have been published recently studying the correlation between PKCα and HCC, and this is a review of the combined results from these papers.

PKC Expression in Human HCC

In a study, we found that PKCα, δ, and ι in HCC tissues were significantly more abundant as compared to non-tumor liver tissues, while no significant difference between HCC tissues and non-tumor liver

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tissues was observed in other isoenzymes (39). We took a look at the survival rate of post-surgery patients and found that patients who showed a low level of PKCα expression were able to survive for significantly longer time than patients with higher PKCα expressions. In immunohistochemistry studies, it was confirmed that above-normal PKCα levels can be found in human HCC (30, 42). It can therefore be suggested that PKCα is a good candidate as a poor prognosis marker in HCC. Another investigator has reported that PKCι is obviously higher in hepatoma than in adjacent normal tissues, and has a positive correlation with the expression of Cyclin E, differentiation degree, and invasion of tumor. It suggests that PKCι expression plays an important role in cell invasion and metastasis of HCC (34). Due to the above findings, PKCα and ι were therefore the primary research subjects in some of the researches of this review, and will be discussed upon in the next section.

The Influence of PKCα on Cell Proliferation, Migration, and Invasion in HCC Cells

Clinical data from our laboratory indicated that PKCα and ι are poor prognosis markers. A study of PKCα and ι involvement in HCC development was undergone through an evaluation of the role of these isoforms in human HCC by investigating 5 human hepatocellular carcinoma cell lines including the poorly differentiated HA22T/VGH and SK-Hep-1, and the well-differentiated PLC/PRF/5, Hep3B, and HepG2 (38). Through the detection of mRNA and protein levels of the 5 cultured cell lines, it was found that mRNA and protein levels in PKCα and ι were significantly increased in poorly differentiated cell lines. In other isoenzymes, no significant changes between the poorly differentiated and well differentiated groups were found.

Further testing yielded the result that treating SK-Hep-1 HCC with antisense PKCα significantly suppressed cell growth, cell migration and invasion (38). It was also able to inhibit cell proliferation in the G1 phase and decrease the expression of Cyclin D1 but increase the expression of p21 and p53. To find out the role of a certain gene in cancer cells, we constructed a short hairpin RNA (shRNA) vector and tested it for PKCα expression inhibition. We found that 5 micrograms of shRNA PKCα vector can completely inhibit PKCα expression in a cell (38), and, in the shRNA stable clones used, the potential of cell growth, migration, and invasion were decreased. The same results were reproduced using PKCα/β inhibitor Go6976, which was also able to significantly inhibit proliferation, migration, and invasion in poorly differentiated HCC cells. Taken together, in human HCC cells, the expression of PKCα protein is related to cell proliferation, migration, and invasion.

PKCα/p38 MAPK Signaling Pathway in HCC Cells

The mitogen-activated protein kinase (MAPK) pathway is relevant to human carcinogenesis (1, 27, 37). There are three mammalian MAPK subfamilies: extracellular signal-regulated kinases (ERK), Jun NH2-terminal kinases (JNK), and p38 kinases. They mediate a variety of signals for cellular functions (13). Activation of MAPK has been associated with PKCα as well as observed in a number of tumors (6, 25, 28, 31, 33).

By measuring the expression of MAPK, we found that p38 MAPK plays an important role in liver malignant tumor progression (26). In PKCα deficient stable clone HCC cells treated with shRNA PKCα, and in antisense PKCα treated HCC cells, p38 MAPK was decreased. Moreover, p38 MAPK inhibitor SB203580 and the dominant negative p38 MAPK were able to inhibit p38 MAPK activation and cell migration and invasion. Further tests by Hsieh *et al.* (9, 10) found that p38 MAPK specific activator MAPK kinase-6 (MKK-6) was able to enhance cell migration and invasion in PKCα deficient stable clone HCC cells. When the MKK-6 treated cells were co-treated with p38 MAPK inhibitor, the enhancement of cell migration and invasion was inhibited. Furthermore, the PKCα deficient stable clone HCC cells treated with dominant-positive mutant PKCα yielded a reverse of PKCα activity and its downstream effects. These findings suggest that PKCα over-expression may be the cause of p38 MAPK activation, and the promoter of cell migration and invasion in poorly differentiated HCC cells.

Contrary to these findings, other researchers have found that interference of the p38 MAPK pathway with inhibitor SB203580 can markedly decrease TGF-β1- or Naphtho[1,2-b] furan-4,5-dione(NFD)-induced cell apoptosis in Hep3B cells, suggesting that the signaling of p38 MAPK can cause apoptosis in Hep3B cells (4, 14). Moreover, transfection of active MKK-6 to HepG2 cells can induce apoptosis through cytochrome c release and caspase-3 activity increase (11), and, in HCC patients, p38 MAPK activity was found significantly lower in larger tumors than that in the smaller tumors, suggesting that p38 MAPK activation may be correlated to cancer cell apoptosis. However, p38 MAPK pathway does play a crucial role in microRNA miR-17-5p-induced phosphorylation of heat shock protein 27 (HSP27) which, as a consequence, can enhance the migration of HCC cells (40). These conflicting results suggest that further research must be continued for elucidating the role

of p38 MAPK in HCC development.

PKCα Signaling Pathway and Invasion-Related Genes MMP-1 and u-PA in HCC Cells

Among the invasion-related genes, the researchers have found that the expression of matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-3 (MMP-3), urokinase-type plasminogen activator (uPA), urokinase-type plasminogen activator receptor (uPAR), and focal adhesion kinase (FAK) are significantly higher in poorly differentiated HCC cells as compared to those in well differentiated HCC cells (38). In addition, in poorly differentiated SK-Hep-1 cells treated with antisense PKCα and PKCα deficient stable clone HCC cells, the expressions of MMP-1, uPA, uPAR, and FAK are decreased.

Furthermore, when we treated SK-Hep-1 cells with p38 inhibitor SB203580 and p-38 dominant-negative vector, it was found that the expressions of MMP-1 and uPA were decreased, but the expressions of uPAR and FAK did not change (results pending publication). We also found that the treatment of PKCα deficient stable clone HCC cells with p38 activator MKK-6 increased the expression of uPA and MMP-1 and that treatment of these transfected cells with SB203580 reversed the elevated expression of uPA and MMP-1 (results pending publication). These results suggest that the promotion of cell migration and invasion by PKCα may be related to the expression of MMP-1 and uPA in HCC cells

PKCα Expression and the Transcription Factors MZF-1 and Elk-1 in HCC Cells

Since PKC levels are high in human HCC and in poorly differentiated human HCC cell lines, to explore the cause of PKCα over-expression in poorly differentiated HCC cells would be a relevant investigation. By ruling out gene amplification and the increase in mRNA stability as the possible causes of over-expression of PKCα in poorly differentiated HCC cells, we deduced that the increase in mRNA transcription is a possible route of investigation (9).

After using antisense of Ets-like protein-1 (Elk-1) and myeloid zinc finger-1 (MZF-1) to treat cells, we found that the protein expressions of the two genes were inhibited, and either antisense can also inhibit PKCα expression (10) as well as decrease the potential of cell migration and invasion. This indicates that Elk-1 and MZF-1 can not only regulate PKCα expression but also promote migration and invasion.

Furthermore, an EMSA assay and a ChIP assay indicated that Elk-1 and MZF-1 can bind to PKCα promoter (10). Also, when transfected the HepG2 with Elk-1 and MZF-1 expression vectors, expressions

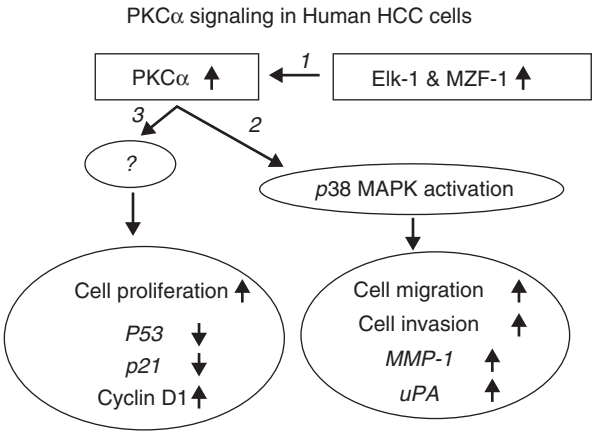

Fig. 1. PKCα singaling in both down-stream and up-stream pathway in human HCC cells. [1] PKCα expression is regulated by Elk-1 and MZF-1 transcription factors, [2] PKCα regulates cell migration and invasion *via* the activation of p38 MAPK, and [3] PKCα regulates cell proliferation through unknown pathway. PKCα, protein kinase C alpha; Elk-1, Ets-like protein-1; MZF-1, myeloid zinc finger-1; p38 MAPK, p38 mitogen-activated protein kinase; MMP-1, matrix metalloproteinase-1; uPA, urokinase-type plasminogen activator.

of ELK-1 and MZF-1 were increased and PKCα expression was dose-dependently increased. In an animal study on tumorigenesis, we found that the mass of tumors treated with in MZF-1 or Elk-1 antisense became smaller than the mass of sense-treated tumors (9, 41). Therefore, these data suggest that MZF-1 and Elk-1 may regulate PKCα expression through transcriptional activation and subsequently promote tumorigenesis.

Conclusion

The following conclusions can be drawn, and are illustrated in Fig. 1: [1] PKCα expression is regulated by Elk-1 and MZF-1 transcription factors in human HCC cells, [2] PKCα regulates human HCC cell migration and invasion *via* the activation of p38 MAPK, and [3] PKCα regulates human HCC cell proliferation through a yet-to-be-found pathway. Also, what mechanism PKCα uses to cause cell proliferation and to alter the expression of p53, p21, and Cyclin D1, whether many other cancers present high levels of PKCα, and whether they are also correlated to MZF-1 and Elk-1, remain to be answered. Our preliminary data has shown that not all cancers could fully support the hypotheses proposed here.

Our further research into MZF-1 and Elk-1 protein interaction has yielded the following results: generally speaking, in tumor cells, when MZF-1 and Elk-1 bind together and then bind to their binding site on the PKCα promoter, PKCα expression is stimu-

lated. When the cells are transfected with DNA binding domain truncated MZF-1, this gene will bind to a wild type of Elk-1. This phenomenon may cause the decrease in the number of successful wild type MZF-1 and wild type Elk-1 interactions and also decrease those binds to the PKCα promoter and lower PKCα expression. A similar effect was observed in cells transfected with DNA binding domain truncated Elk-1.

In terms of cancer therapeutics, PKCα has been a target for the drug aprinocarsen (ISIS 3521), which has been studied as a single agent, as well as in combination with standard chemotherapeutics in cancer patients in over 20 trials from phase I to phase III (8, 18). However, many difficult situations still prevent a breakthrough (19). Therefore, based on this review of PKCα expression regulated by two transcription faction factors, *i.e.* MZF-1 and Elk-1, and that transcription regulation is deemed a hopeful strategy for cancer treatment (5), further research on transcription regulation in the upstream of PKCα gene expression may be an ideal way to increase the knowledge about the regulation of PKCα gene expression in human cancer.

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