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Antisense expression of protein kinase Cα improved sensitivity to anticancer drugs in human lung cancer LTEPa-2 cells[1]

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KEY WORDS protein kinase C; lung neoplasms; cultured tumor cells; MDR genes; multiple drug resistance; antisense RNA; Northern blotting; Western blotting; doxorubicin; harringtonines

AIM: To study the role of protein kinase Cα (PKCα) in sensitivity to some clinical anticancer drugs in human lung cancer LTEPa-2 cells. **METHODS**: Human lung cancer cell model expressing antisense PKCα was established and characterized by gene transfection and immunoblotting. Northern blotting was used to analyze the expression of multiple drug resistance (*mdr*-1) gene and antisense PKCα mRNA. IC_{50} for some anticancer drugs in cultured cells were measured. **RESULTS**: Expression of antisense PKCα mRNA inhibited *mdr*-1 gene expression in lung cancer cells and improved sensitivity to anticancer drugs (harringtonine, carboplatin, bleomycin A_5, vincristine and doxorubicin) in lung cancer cells. IC_{50} for harringtonine, carboplatin, bleomycin A_5, vincristine, and doxorubicin was decreased by 46.4 %, 42.1 %, 79 %, 69.9 %, and 61.6 % respectively. **CONCLUSION**: PKCα plays an important regulation role of *mdr*-1 gene expression and drug sensitivity in human lung cancer cells.

The sensitivity of cancer cells to chemotherapeutic agents may be greatly influenced by intracellular signaling systems such as protein kinase C (PKC). The depletion of PKC was necessary for sensitization of tumor cells to anticancer agents, combination of PKC inhibitor quercitin and cDDP significantly reduced the growth of human large-cell lung tumor in nude mice[1]. A novel approach is to increase the sensitivity of tumor tissue by agents which affect signal transduction pathways[2]. This paper was to study the sensitivity of human lung cancer cells to some antitumor drugs using constructed cell model expressing antisense PKCα.

MATERIALS AND METHODS

Reagents The expression vectors pXJ41-neo were generously provided by Institute of Molecular and Cell Biology, National University of Singapore. Recombinant plasmids pXJ41-CKPα containing PKCα cDNA in antisense orientation were constructed in our Lab. Harringtonine, carboplatin, bleomycin A_5, vincristire, and doxorubicin were purchased from Sigma and dissolved in distilled PBS (pH 7.4).

Cell culture Human lung cancer cells LTEPa-2 were kindly provided by Prof FAN Mu-Zheng (Sino-Japan Friendship Hospital). Control cells (LTC1) were transfected with vector pXJ41-neo only. LTEPa-2 cells were transfected with plasmids pXJ41-CKPα containing the neo resistance gene for selection in G418 and an antisense cDNA for PKCα under the control of the human cytomvirus promoter (Fig 1). All cell lines were maintained in DMEM (Life Technologies, Inc) supplemented with 10 % calf serum in a 5 % CO_2 incubator at 37 ℃.

Western blot Cell lysates were separated on 8 % SDS-PAGE and blotted onto nitrocellulose filter. Blots were probed with rabbit polycolonal antibody against PKCα isoform (Gibco-BRL). Immunoreactive protein was visualized with goat anti-rabbit IgG conjugated to horseradish peroxidase, with DAB (3′, 3′-diaminobenzidene, Sigma) as the substrates.

Assay for PKC activity Logarithmic phase cells were harvested 3 d after seeding and cell extracts were assayed for PKC activity[3].

Northern blot Total RNA was isolated by the method[4]. Total RNA 30 μg from each cell type was electrophoresed on a 1.2 % formaldehyde gel and transferred to nitrocellulose. Loading of equivalent quantities of RNA per lane was verified by expression of β-actin gene. The

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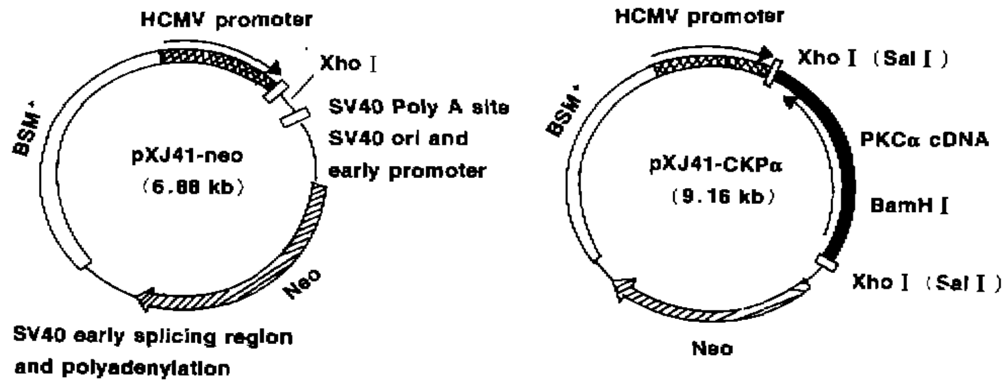


Fig 1. Map of pXJ41-neo and pXJ41-CKPα.

blots were hybridized to [^{32}P]-labeled sense RNA for PKCα and random hexamer-primed *mdr*-1 cDNA. Filters were then washed in 2 × SSC – 1 % SDS at 37 ℃, 0.1 × SSC – 0.1 % SDS at 52 ℃ respectively, and exposed to X-ray film at – 80 ℃.

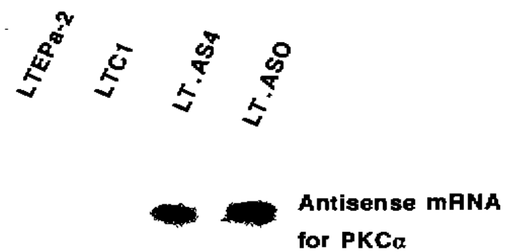
Cell proliferation 2×10^4 cells were seeded on 24-well microplates and incubated for 24 h with DMEM medium containing the drugs. Cells were trypsinized and counted after 72 h by coulter counter. IC_{50} value of each drug was calculated by weighted probit analysis.

RESULTS

PKCα level in LTEPa-2 cells expressing antisense PKCα PKCα protein content (Fig 2) and Ca^{2+}/phosphatidylserine-dependent PKC activity were reduced apparently in LT.AS4 cells stably expressing antisense RNA for PKCα in contrast to control cells (LTC1) transfected with pXJ41-neo only. The PKC activity in LT.AS4, LTC1 and lung cancer LTEPa-2 cells was 8.4 ± 0.3 Bq/g protein · min^{-1}, 31.5 ± 0.3 Bq/g protein · min^{-1}, and 33.4 ± 0.6 Bq/g protein · min^{-1} respectively. This result showed that the PKC activity was decreased by 73.3 % and 74.8 % in LT.AS4 cells expressing antisense PKCα in comparison with control cell lines (LTC1) and parent cells (LTEPa-2), respectively.

Sensitivity of LTEPa-2 cells expressing antisense PKCα to chemotherapeutic agents Antisense expression of PKCα improved sensitivity of human lung cancer cells (LTEPa-2)

Expression of antisense mRNA for PKCα in LTEPa-2 cells expressing antisense PKCα



Expression of PKCα protein in LTEPa-2 cells expressing antisense PKCα

Fig 2. PKCα level in human lung cancer LTEPa-2 cells expressing antisense PKCα.

to some antitumor drugs such as harringtonine, carboplatin, bleomycin A_5, vincristine, and doxorubicin (Tab 1). In LT.AS4 cells transfected antisense PKCα, IC_{50} for doxorubicin, bleomycin A_5 and harringtonine was decreased by 61.6 %, 79 %, and 46.4 % respectively *vs* control cells (LTC1) transfected with vector only.

***mdr*-1 Gene expression in LTEPa-2 cell expressing antisense PKCα** Expression of *mdr*-1 gene was decreased in cells expressing antisense PKCα compared with control cells and parent cells (Fig 3).

Fig 3. Expression of *mdr*-1 gene in human lung cancer LTEPa-2 cells expressing antisense PKCα.

DISCUSSION

In this work, inhibition of PKCα by antisense method improved the sensitivity of LTEPa-2 cells to some antitumor drugs, the experiment provided the first evidence that IC_{50} of herringtonine decreased by 46.4 % in LT.AS4 cells *vs* control cells. The results further supported that the cellular signaling events regulated the sensitivity of cancer cells to chemotherapeutic agents[5].

It is well known that one factor that causes cancer chemotherapy to fail is the development of multiple drug resistance (MDR). It is believed that product of *mdr*-1 gene expression, P-glycoprotein functions as an active drug efflux pump and extrudes drug from cells below therapeutic level[6]. Many lines of evidence implicate PKC in the regulation of the MDR phenotype[7-11]. The most direct evidence for the role of PKC as a resistance mechanism was that overexpression of PKCα in cells expressing P-glycoprotein was able to enhance the MDR phenotype[12, 13]. These studies defined a particular role for PKCα in modulating the MDR phenotype. In present work, expression of *mdr*-1 gene decreased in human lung cancer cells expressing antisense PKCα. The promoter region of the Chinese hamster P-170 gene contains AP-1 binding site[14]. Furthermore, we found that expression of antisense PKCα decreased AP-1 DNA binding activity (data not shown), this is the mechanism by which PKCα affects expression of *mdr*-1 gene. In conclusion, the results of the present investigation reveal a connection between drug sensitivity and PKC signal transduction pathway in human lung cancer cell (LETPa-2) and suggest that PKCα is an important target for chemotherapy in treatment of tumors.

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Tab 1. Effects of antisense PKCα expression on the sensitivity of LTEPa-2 cells to some chemotherapeutic agents.

Drugs	IC_{50} (95 % confidence limits) LTEPa-2	LTC1	LTAS4	Reduction of IC_{50}/%
Doxorubicin/μg·L⁻¹	28.5 (24.1-34.2)	32.3 (27.4-40.5)	12.4 (9.6-16.4)	61.6
Harringtonine/mg·L⁻¹	0.13 (0.11-0.15)	0.15 (0.13-0.17)	0.08 (0.07-0.10)	46.4
Bleomycin/mg·L⁻¹	1.8 (1.5-2.1)	2.4 (2.2-2.6)	0.5 (0.3-0.7)	79.0
Vincristine/mg·L⁻¹	6.2 (4.6-7.1)	7.3 (4.7-9.9)	2.2 (1.1-3.4)	69.9
Carboplatin/mg·L⁻¹	13.6 (11.3-16.4)	12.6 (9.0-16.9)	7.3 (5.5-9.8)	42.1

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表达反义蛋白激酶 Cα 提高人肺癌 LTEPa-2 细胞对抗肿瘤药物的敏感性[1]

R734.205

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关键词 蛋白激酶 C；肺肿瘤；培养的肿瘤细胞；MDR 基因；多种抗药性；反义 RNA；RNA 印迹；蛋白质印迹；阿霉素；三尖杉酯碱类 抗肿瘤

目的：研究蛋白激酶 Cα (PKCα)在人肺癌 LTEPa-2 细胞对一些临床抗肿瘤药物敏感性中的作用. **方法**：通过基因转染，免疫印迹等方法建立表达反义 PKCα 的人肺癌细胞模型，Northern 印迹检测多药抗性基因的表达，分析了几种抗癌药物对培养细胞的 IC_{50}. **结果**：表达反义 PKCα RNA 降低胞内 PKCα 水平时可抑制肺癌细胞中多药抗性基因的表达，增强肺癌细胞对抗肿瘤药物(三尖杉酯碱、卡铂、博来霉素、长春新碱、阿霉素)的敏感性. **结论**：PKCα 在人肺癌细胞多药抗性基因的表达以及药物敏感性中具有重要调节作用.

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