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蛋白激酶 D1 (PKD1) 在 HIV-1 复制中的作用

The Role of Protein Kinase D 1 in HIV-1 Replication

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CERTIFICATION

I Professor Chen Ruichuan (陈瑞川), hereby certify that I have read this manuscript and recommend for acceptance by the Xiamen University a dissertation entitled “The Role of Protein Kinase D 1 in HIV-1 Replication” in fulfillment of degree of Master of Science of Xiamen University.

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DEDICATION

To my dear mum **Madam Catherine Aweya** who passed away on March 11, 2009 whiles I was working on this thesis.

Mum, you taught me all the good things about life which have thus seen me this far. May your soul and all other departed souls of the family rest in perfect peace.

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Abstract

The Protein kinase D (PKD) enzymes, which comprise of three isoforms (PKD1/PKD μ , PKD2 and PKD3/PKD ν), are generally localized to the cytosol, but in stimulated cells, they are translocated to various subcellular compartments. These enzymes play numerous roles in a variety of normal and pathological processes including transcriptional response to mitochondrial oxidative stress, T-cell receptor signaling, cardiac gene expression and contractility, apoptosis and carcinogenesis. They are also involved in a number of cellular processes such as regulation of Golgi organisation, protein trafficking, Golgi vesicle fission, and are at the converging point of a number of signal pathways.

In this study, we showed that although PKD1 is not expressed in HeLa cells, it could be ectopically expressed and was able to stimulate HIV luciferase activity. However, using deletions mutants of the HIV-1 LTR, we showed that induced expression of HIV-1 by PKD1 did not require TAR and did not also depend on the HIV LTR promoter. On the other hand, in 293T cells, knockdown of PKD1 resulted in the inhibition of HIV-1 expression. Similarly, we also showed that PKD1 did not activate P-TEFb, suggesting that PKD1 may not influence transcription elongation step. Finally, using qRT-PCR tool, we found that overexpression of PKD1 did not influence the mRNA level of luciferase, suggesting that PKD1 had no effect on RNA transcription.

Taken together, by transient transfected system, our results suggest that PKD1 may influence post-transcription steps and may also induce the accumulation of proteins. These findings therefore suggests some probable novel function (s) of PKD1 in cardiac hypertrophy.

Keywords: PKD; HIV-1; Gene expression; Cardiac hypertrophy

摘 要

PKD 家族由 PKD1, PKD2h 和 PKD3 组成, 通常主要分布在胞浆, 但在刺激条件下, 可转位到各亚细胞结构。这些激酶在生理和病理过程中, 具有广泛而重要的作用。包括线粒体氧化应激, T 细胞受体信号, 心肌基因表达和心肌收缩, 细胞凋亡和肿瘤发生等。它们的功能还涉及许多细胞生物学过程, 例如高尔基体组织, 蛋白运输, 高尔基体囊泡分泌以及其他各种细胞信号途径等。

本研究中, 我们发现, HeLa 细胞虽然不表达内源性 PKD1, 但却能异位表达外源导入的 PKD1, 并且可以刺激 HIV-LTR 荧光虫酶报告基因的蛋白表达。然而对 HIV-LTR 启动子进行各种删除突变, 并不能抑制 PKD1 所刺激的 HIV-LTR 荧光虫酶报告基因的蛋白表达。另一方面, 在 293T 细胞中, PKD1, PKD2 和 PKD3 均能正常表达, 当采用 RNAi 技术抑制 PKD1 表达时, 可抑制 HIV-LTR 荧光虫酶报告基因的蛋白表达, 从另一个侧面证明 PKD1 对 HIV-LTR 报告基因蛋白表达的影响。此外, 我们的结果证明: PKD1 不能激活转录延伸过程中最重要的调控因子—正性转录延伸因子 b 的活性, 提示 PKD1 不能影响基因转录的延伸过程。最后我们的定量 RT-PCR 检测结果显示: PKD1 虽然能够激活 HIV-LTR 荧光虫酶报告基因的蛋白表达, 但不能刺激 HIV-LTR 荧光虫酶报告基因 mRNA 的转录。

综上所述, 采用瞬转体系, 我们的结果提示, PKD1 可能影响了转录后的步骤, 并且可能导致蛋白的积累。因此, 我们的研究提示, PKD1 在心肌肥大中, 可能还有其他新的功能。

关键词: PKD; HIV-1; 基因表达, 心肌肥大

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Abbreviations and Acronyms

14-3-3 proteins	Family of conserved regulatory molecules expressed in eukaryotes
AGC	cAMP-dependent, cGMP-dependent and Protein kinase C
AIDS	Acquired immune deficiency syndrome
AMPK	5' Adenosine monophosphate-activated protein kinase
APS	Ammonium persulfate
ART	Antiretroviral therapy
BCR	B-cell receptor
Ca ²⁺ /CaMK	Calcium/Calmodulin-dependent kinase
CaCl ₂	Calcium chloride
cAMP	cyclic adenosine monophosphate
CCKB	Cholecystokinin B receptor
CDK9	Cyclin-dependent kinase 9
cDNA	complementary DNA
CO ₂	Carbon (IV) oxide or carbon dioxide
CRD	Cysteine-rich domain
CRM1	Chromosomal region maintenance protein 1
DAG	Diacylglycerol
DEPC	Diethylpyrocarbonate
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EGFP	Enhanced Green Fluorescent Proteins
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
FBS	Fetal Bovine Serum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase

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