学校编码：10384　　分类号＿＿＿密级＿＿

学号：21720071154108　　UDC ＿

厦门大学

硕　士　学　位　论　文

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

By

Aweya Jude Juventus

指导教师姓名:Prof. Chen Ruichuan (陈瑞川)

专　业　名　称: 生物化学与分子生物学

论文提交日期: 2009 年 5 月

论文答辩时间: 2009 年 6 月

学位授予日期: 2009 年　月

答辩委员会主席：＿＿＿＿＿＿

评　　阅　　人：＿＿＿＿＿＿

2009 年　月

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.

Signed Supervisor

Date

School of Life Sciences
Xiamen University
Xiamen, Fujian
P.R. China

厦门大学学位论文原创性声明

兹呈交的学位论文，是本人在导师指导下独立完成的研究成果。本人在论文写作中参考的其他个人或集体的研究成果，均在文中以明确方式标明。本人依法享有和承担由此论文而产生的权利和责任。

声明人（签名）：

2009 年　　月　日

DECLARATION

I here declare that apart from the sources cited, this dissertation is my own work under the supervision of my supervisor Professor Chen Ruichuan (陈瑞川). The materials of the dissertation have not been presented and it will not be presented to any other university other than this univerity for similar or any other degree.

Signature:

Candidate: **Aweya Jude Juventus**

厦门大学学位论文著作权使用声明

本人完全了解厦门大学有关保留、使用学位论文的规定。厦门大学有权保留并向国家主管部门或其指定机构送交论文的纸质版和电子版，有权将学位论文用于非赢利目的的少量复制并允许论文进入学校图书馆被查阅，有权将学位论文的内容编入有关数据库进行检索，有权将学位论文的标题和摘要汇编出版。保密的学位论文在解密后适用本规定。

本学位论文属于

1、保密（　），在　　年解密后适用本授权书。

2、不保密（　）

（请在以上相应括号内打“√”）

作者签名：　　日期：2009 年 5 月 15 日

导师签名：　　日期：　　年　月　日

COPYRIGHT

Signature:

Candidate: **Aweya Jude Juventus**

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.

Abstract

The Protein kinase D (PKD) enzymes, which comprise of three isoforms (PKD1/PKDμ, PKD2 and PKD3/PKDυ), 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 overexpresion 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—正性转录延升因子 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

Table of Contents

目录

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
Ca2+/CaMK	Calcium/Calmodulin-dependent kinase
CaCl2	Calcium chloride
cAMP	cyclic adenosine monophosphate
CCKB	Cholecystokinin B receptor
CDK9	Cyclin-dependent kinase 9
cDNA	complementary DNA
CO2	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