

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการการวิจัยแห่งชาติ



E47346

# THE ROLE OF ADAPTOR PROTEIN NCK1 IN T CELL ACTIVATION AND FUNCTION

ICHAYA YIEMWATTANA

*A Thesis Submitted to the Graduate School of Naresuan University  
in Partial Fulfillment of the Requirements  
for the Doctor of Philosophy Degree in Oral Biology*

*April 2012*

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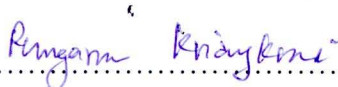
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This thesis entitled "The Role of Adaptor Protein Nck1 in T Cell Activation and Function" submitted by Ichaya Yiemwattana in partial fulfillment of the requirements for the Doctor of Philosophy Degree in Oral Biology is hereby approved.

  
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ACTIVATION AND FUNCTION

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### ABSTRACT

**E17346**

The non-catalytic region of tyrosine kinase (Nck) is proposed to play an essential role in T cell activation. However, evidence based on functional and biochemical studies has brought into question the critical function of Nck. Therefore, the aim of the present work was to investigate the role of Nck in T cell activation. To study this, the human Jurkat T cell line was used as a model for human T lymphocytes. The short interfering (si) RNA targeting Nck1 gene was used with electroporation to knock-down Nck1 protein expression in Jurkat T cells. The results showed that decreased Nck1 protein expression did not affect the apoptosis of the transfected Jurkat T cells compared with control siRNA-transfected cells and non-transfected cells both in unstimulated or anti-TCR monoclonal antibody (C305) stimulated groups. Upon CD3 $\epsilon$ /CD28 stimulation, knock-down of Nck1 in Jurkat T cells caused a decrease in CD69 expression and in interleukin (IL)-2 secretion. However, no significant alterations of CD69 and IL-2 expression were found upon phytohaemagglutinin (PHA)/phorbol myristate acetate (PMA) stimulation. Knock-down of Nck1 had no effect on the proliferation of Jurkat T cells stimulated with either PHA or anti-TCR antibody. In conclusion, the decreased Nck1 protein in Jurkat T cells resulted in impairment of TCR/CD3-mediated activation and function.

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## ABBREVIATIONS

Ab	=	antibody
TCR	=	T cell antigen receptor
MHC	=	major histocompatibility complex
ITAM	=	immunoreceptor tyrosine-based activation motif
SFK	=	Src family tyrosine kinase
Lck	=	lymphocyte protein -tyrosine kinase
ZAP-70	=	ζ-chain-associated protein 70 kDa
LAT	=	linker for activation of T cells
SLP-76	=	SH-2-domain containing leukocyte protein of 76 kDa
GRB2	=	Growth factor receptor-bound protein 2
GADS	=	Grb2-related adaptor downstream of shc
PLCγ1	=	phospholipase Cγ1
PIP2	=	phosphatidylinositol 4,5-bisphosphate
IP <sub>3</sub>	=	inositol 3,4,5-triphosphate
DAG	=	diacylglycerol
PKC	=	protein kinase C
RasGRP	=	Ras guanyl nucleotide-releasing protein
RasGEF	=	Ras guanine nucleotide exchange factor
SOS	=	son of sevenless
PAK	=	p21-activated kinases
NFAT	=	nuclear factor of activated T cell
Nck	=	non-catalytic region of tyrosine kinase
PRS	=	proline-rich sequence
IL-2	=	interleukin 2
SEB	=	staphylococcal enterotoxin B
SH2	=	Src homology 2
SH3	=	Src homology 3
WASP	=	Wiskott-Aldrich syndrome protein
Arp2/3	=	actin-related proteins 2 and 3
Cdc42	=	cell division cycle 42

## ABBREVIATIONS (CONT.)

WIP	=	WAS-interacting protein
RNAi	=	small RNA interference
siRNA	=	short interfering RNA
RISC	=	RNA-induced silencing complex
RT-PCR	=	reverse transcription-polymerase chain reaction
PHA	=	phytohemagglutinin
PI	=	propidium iodide
SOCS7	=	suppressor of cytokine signal-7
PMA	=	phorbol 12-myristate 13-acetate
AIM	=	activation inducer molecule
NF- $\kappa$ B	=	nuclear factor kappa-light-chain-enhancer of activated B cells
Egr-1	=	Early growth response protein 1
AP-1	=	activating protein-1
CD25	=	IL-2 receptor $\alpha$
pEGFP	=	plasmid enhanced green fluorescent protein
BCA	=	bicinchoninic acid
SDS-PAGE	=	sodium dodecyl sulfate polyacrylamide gel electrophoresis
FITC	=	fluorescein isothiocyanate
BrdU	=	5-bromo-2'-deoxyuridine
TMB	=	tetramethylbenzidine
PBS	=	phosphate-buffered saline
RT	=	room temperature
PE	=	phycoerythrin
SSC	=	side scatter
FSC	=	forward scatter
ELISA	=	Enzyme-linked immunosorbent assay
HIP55	=	HPK1-interacting protein of 55 kDa
FYB	=	Fyn-binding protein