

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการการวิจัยแห่งชาติ



E46240

THE INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS-1
INTEGRATION BY DESIGNED ZINC FINGER PROTEIN

SUPACHAI SAKKHACHORNPHOP

DOCTOR OF PHILOSOPHY
IN BIOMEDICAL SCIENCE

THE GRADUATE SCHOOL
CHANG MAI UNIVERSITY
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**A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN
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IN BIOMEDICAL SCIENCE**

**THE GRADUATE SCHOOL
CHIANG MAI UNIVERSITY**

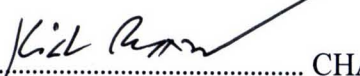
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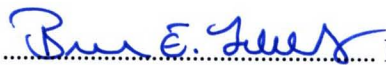
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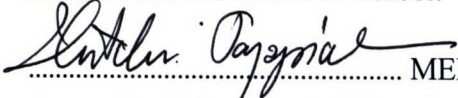
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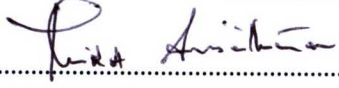
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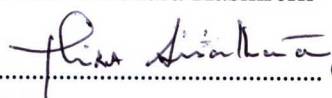

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Supachai Sakkhachornphop

Thesis Title	The Inhibition of Human Immunodeficiency Virus-1 Integration by Designed Zinc Finger Protein	
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ABSTRACT

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Integration of the human immunodeficiency virus type 1 (HIV-1) genome into the host chromosome is a crucial step in the HIV life cycle. The highly conserved cytosine-adenine (CA) dinucleotide sequence immediately upstream of the cleavage site is imperative for integrase (IN) activity. As this viral enzyme has an important role early stage of infection, interference with the IN substrate has become an attractive strategy for therapeutic intervention. In this study, the integrase recognition sequence at the 2-LTR-circle junctions of HIV-1 DNA was used to design a six-contiguous zinc finger protein (ZFP), namely 2LTRZFP by using zinc finger tools. The designed motif fused to green fluorescent protein (GFP) was expressed and purified from *E. coli* to determine its binding properties. The binding affinity of 2LTRZFP-GFP to its target DNA via surface plasmon resonance (SPR) was on a

nanomolar scale. The competitive SPR indicated the 2LTRZFP-GFP specifically interacted with its target DNA. The qualitative binding activity was subsequently determined by electrophoretic mobility shift assay (EMSA) and demonstrated the aforementioned correlation. To investigate an intracellular function of 2LTRZFP-GFP, the 293T stable line of transduced with 2LTRZFP-GFP was produced and challenged with VSV-G pseudotyped lentiviral red fluorescent protein (RFP). The result demonstrated the dramatic suppression of RFP expression by 2LTRZFP-GFP. In addition, a third-generation lentiviral vector and pCEP4 expression vector were used to deliver the 2LTRZFP-GFP transgene into human T-lymphocytic cells for production of stable cell lines in long-term expression studies. These cell lines were challenged with HIV-1_{NL4-3}. 2LTRZFP-GFP successfully inhibited viral integration and replication as measured by an *Alu-gag* qPCR and p24 antigen assay, respectively. These findings indicated that viral integration can be inhibited by intracellular immunization with 2LTRZFP-GFP indicating its potential for use in HIV gene therapy.

การยับยั้งการอินทิเกรตเชื้อ เอชไอวี-1 โดยซิงก์ฟิงเกอร์โปรตีน
ที่ออกแบบ

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กระบวนการอินทิเกรชันของเอชไอวี-1 คือเอ็นเอ เข้าสู่โครโมโซมเจ้าบ้านโดยเอนไซม์อินทิเกรส เป็นขั้นตอนที่สำคัญในระยะแรกสำหรับการแบ่งตัวของไวรัส เอนไซม์อินทิเกรสทำการตัดดีเอ็นเอเป้าหมายบริเวณปลายสามไพร้มของแอลทีอาร์ เพื่อเตรียมขึ้นดีเอ็นเอเข้าไปเชื่อมต่อกับดีเอ็นเอเจ้าบ้าน ดังนั้นการรบกวนหรือยับยั้งไม่ให้อินทิเกรสทำงานได้จึงเป็นกลยุทธ์ที่น่าสนใจในการรักษาในระดับยีน ในการศึกษาที่ผู้วิจัยได้ใช้โมเลกุลเป้าหมายคือ ดีเอ็นเอบริเวณรอยต่อของสองแอลทีอาร์ของเชื้อไวรัสเอชไอวี-1 เป็นต้นแบบในการออกแบบซิงก์ฟิงเกอร์โปรตีนที่จำเพาะ ซิงก์ฟิงเกอร์โปรตีนที่ออกแบบจากซิงก์ฟิงเกอร์ทูต มีจำนวน 6 ฟิงเกอร์มีชื่อว่า 2LTRZFP โปรตีนนี้ถูกเชื่อมต่อกับโปรตีนที่เรืองแสงสีเขียว (GFP) ซึ่งสามารถเตรียมได้จากแบคทีเรียอีโคไล และทำให้บริสุทธิ์เพื่อใช้ในการทดสอบการเข้าจับกันระหว่างซิงก์ฟิงเกอร์โปรตีนและดีเอ็นเอเป้าหมาย โดยวิธีเซอร์เฟสพลาสมอนเรโซแนนซ์ (SPR) โดยได้ค่าคงที่ในการเข้าจับในระดับนาโนโมลาร์ ในขณะที่วิธี คอมเพทิทีฟเซอร์เฟสพลาสมอนเรโซแนนซ์ชี้ให้เห็นว่า 2LTRZFP-GFP จับกับดีเอ็นเอเป้าหมายอย่างจำเพาะ การเข้าจับกันเชิงคุณภาพระหว่างซิงก์ฟิงเกอร์โปรตีนและดีเอ็นเอเป้าหมายถูกทำการทดสอบโดยวิธีอิเล็กโตรโมบิลิตีซีฟแอสเซ (EMSA) ผลการทดลองที่ได้มีความสอดคล้องกับวิธีเซอร์เฟสพลาสมอนเรโซแนนซ์ ดังกล่าว เพื่อศึกษาหน้าที่ของ 2LTRZFP-GFP ภายในเซลล์เซลล์ 293T ถูกนำส่งยีน 2LTRZFP-GFP โดยไวรัสเพื่อสร้างเซลล์ที่มีการแสดงออกของ 2LTRZFP-GFP อย่างถาวร และทำการทำลายด้วยเชื้ออีเอสวี-จี ซูโคโทปเอนติไวรัสที่มีโปรตีนเรืองแสงสีแดง (RFP) ผลการทดลองพบว่า 2LTRZFP-GFP ลดการแสดงออกของโปรตีนเรืองแสงสีแดง (RFP) ได้ชัดเจน ยิ่งไปกว่านั้น เอนติไวรัสเวกเตอร์และพีแซฟโฟเวกเตอร์ถูกนำมาใช้ในการนำส่งยีน 2LTRZFP-GFP เข้าในเซลล์เพาะเลี้ยงชนิดที-ลิมโฟไซต์ของมนุษย์เพื่อทำการสร้างเซลล์ที่มีการ

แสดงออกของ 2LTRZFP-GFP อย่างถาวร เซลล์เหล่านี้ถูกทำลายด้วยเชื้อเอชไอวี-วัน เอ็นแอลพีว-
ทรี พบว่า 2LTRZFP-GFP ยับยั้งกระบวนการอินทิเกรชันและการแบ่งตัวของไวรัสได้สำเร็จโดยวัด
จาก *Alu-gag* qPCR และ p24 antigen assay ตามลำดับ การค้นพบนี้แสดงให้เห็นว่ากระบวนการ
อินทิเกรชันของไวรัสสามารถถูกยับยั้งได้โดย 2LTRZFP-GFP ในเซลล์ ซึ่งแนวความคิดใหม่นี้เป็น
การสร้างนวัตกรรมการรักษาผู้ติดเชื้อเอชไอวีในระดับยืนต่อไปในอนาคต

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ABBREVIATIONS AND SYMBOLS

%	Percent
α	Alpha
β	Beta
Ψ	Psi-sequences
°C	Degrees Celsius
AIDS	Acquired immunodeficiency syndrome
<i>Alu</i>	<i>Arthrobacter luteus</i>
BBQ	BlackBerry Quencher
bp	Base pair (s)
BSA	Bovine serum albumin
CA	Capsid protein
CCR5	C-C chemokine receptor type 5
CD4	Cluster of differentiation 4
cDNA	Complementary DNA
CMV	Cytomegalovirus
cPPT	Central polypurine tract
C_t s	Cycle thresholds
CXCR4	C-X-C chemokine receptor type 4
Cys	Cysteine
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle's medium

DNA	Deoxyribonucleic acid
ds-DNA	Double-stranded DNA
DTT	Dithiothreitol
EBV	Epstein-Barr virus
<i>E. coli</i>	<i>Escherichia coli</i>
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EMSA	Electrophoretic mobility shift assay
ECL	Enhanced Chemiluminescence
ENV	Envelope
ERD	ERF repressor domain
FAM	6-carboxyfluorescein
FBS	Fetal bovine serum
FP	Fusion protein
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GAG	Group specific antigens
GFP	Green fluorescent protein
gm	Gram (s)
HAART	Highly active antiretroviral therapy
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hr	Hour (s)
HIV	Human Immunodeficiency virus-1
His	Histidine
HCl	Hydrochloric acid

HRP	Horseradish peroxidase
HSCs	Hematopoietic stem cells
IN	Integrase
IPTG	Isopropyl β -D-thiogalactopyranoside (IPTG)
kb	Kilo base pair (s)
KCl	Potassium chloride
Kd	Dissociation constant
kDa	Kilodaltons
KRAB	Kruppel associated box
LB	Luria-Bertani
Leu	Leucine
LTR	Long terminal repeat
M	Molar (s)
MA	Matrix protein
mAb	Monoclonal antibody (-ies)
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
MgCl ₂	Magnesium Chloride
min	Minute (s)
ml	Milliliter (s)
MOI	Multiplicity of infection
mPGK	Murine phosphoglycerate kinase
Mw	Molecular weight
NaCl	Sodium chloride

NaN ₃	Sodium azide
NaOH	Sodium hydroxide
NC	Nucleocapsid protein
NEF	Negative Regulatory Factor
ng	Nanogram (s)
NLS	Nuclear localization signal
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
nm	Nanometer (s)
nM	Nanomolar (s)
NRTIs	Nucleoside/nucleotide reverse transcriptase inhibitors
OD	Optical density
OriP	Origin of replication
PBS	Phosphate buffered saline
PBS	Primer binding site
PCR	Polymerase chain reaction
Phe	Phenylalanine
pI	Isoelectric point
PICs	Preintegration complexes
PMSF	Phenylmethylsulfonylfluoride
POL	Polymerase
PPT	Polypurine tract
PRIs	Protease inhibitors
qPCR	Quantitative polymerase chain reaction
RCL	Replication competent lentiviruses

RFP	Red fluorescent protein
RNA	Ribonucleic acid
rpm	Revolutions per minute
RRE	Rev-responsive element
RT	Reverse transcriptase
S	Second (s)
scFv	Single-chain variable fragments
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SIN	Self-inactivating
siRNAs	Small interfering RNAs
shRNAs	Short hairpin RNAs
SPR	Surface plasmon resonance
ss-DNA	Single-stranded DNA
TAT	Transactivator of transcription
TBP	TATA-box binding protein
TSO	Target site overlap
Tyr	Tyrosine
μg	Microgram (s)
μl	Microliter (s)
μm	Micrometer (s)
μM	Micromolar (s)
V	Volt (s)

VIF	Viral infectivity factor
VPR	Viral protein R
Vpu	Viral protein U
VSV-G	Vesicular stomatitis virus G glycoprotein
V/V	Volume /Volume
WHO	World Health Organization
WPRE	Woodchuck hepatitis virus post-transcriptional regulatory element
W/V	Weight/Volume
ZFP	Zinc finger protein
ZnSO ₄	Zinc sulfate