

SER/THR PHOSPHATASES PP1 γ AND PP2C α IN REGULATING NEURONAL INSULIN SIGNALING AND INSULIN RESISTANCE

YAMINI YADAV



**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES
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**SER/THR PHOSPHATASES PP1 γ AND PP2C α
IN REGULATING NEURONAL INSULIN
SIGNALING AND INSULIN RESISTANCE**

By

**Yamini Yadav
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Kusuma School of Biological Sciences

Submitted

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*Dedicated to
my mother
and
all my teachers*

Certificate

This is to certify that the thesis entitled “**Ser/Thr phosphatases PP1 γ and PP2C α in regulating neuronal insulin signaling and insulin resistance**” being submitted by **Ms. Yamini Yadav** to **Indian Institute of Technology Delhi** for the award of the degree of “**Doctor of Philosophy**” is a record of bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with rules and regulations of Indian Institute of Technology-Delhi, India. The results prescribed in it have not been submitted in part or full to any other University or Institute for the award of any Degree/ Diploma.



Dr. Chinmoy Sankar Dey

Professor

Kusuma School of Biological Sciences

Indian Institute of Technology, Delhi

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A handwritten signature in blue ink that reads "Yamini." with a small horizontal line under the "i".

Yamini Yadav

ABSTRACT

Insulin signaling is well established in tissues like skeletal muscle, adipocytes, hepatocytes etc.; however, insulin signaling in brain is less established. Insulin signaling in brain regulates various functions like glucose homeostasis, memory formation, cognition, energy expenditure etc. Defective insulin signaling leads to insulin resistance in brain which increases the risk of neurodegenerative diseases like Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD) etc.

Insulin signaling is phosphorylation/dephosphorylation driven cascade and regulated by the balanced actions of kinases and phosphatases. In the past years attempts have been made to elucidate the role and involvement of few kinases in neuronal insulin signaling and insulin resistance. However, phosphatases were always given less attention. Based on the amino acid residues they dephosphorylate, phosphatases were broadly divided into three classes: Tyr phosphatases, Ser/Thr phosphatases and Dual specificity phosphatases. Ser/Thr phosphatases are further subdivided into the phosphoprotein phosphatase (PPP) family, which includes PP1, PP2A and PP2B, and the protein phosphatase Mg^{2+} or Mn^{2+} -dependent (PPM) family, which includes PP2Cs. Among which PTEN (Tyr phosphatase) and SHIP2 (Dual specificity phosphatase) have been previously reported as important regulators of neuronal insulin signaling. However, there is no report determining the role of Ser/Thr phosphatases in neuronal insulin signaling and insulin resistance. Therefore, in present study we determined (1) the role of PP2C α (one of the isoforms of PP2Cs) in regulating neuronal insulin signaling and insulin resistance, and (2) the role of PP1 γ (one of the isoforms of catalytic subunit of PP1) in regulating neuronal insulin signaling and insulin resistance.

सारांश

कंकाल की मांसपेशी, एडिपोसाइट्स, हेपेटोसाइट्स आदि जैसे ऊतकों में इंसुलिन सिग्नलिंग अच्छी तरह से स्थापित है; हालाँकि, मस्तिष्क में इंसुलिन सिग्नलिंग कम स्थापित है। मस्तिष्क में इंसुलिन सिग्नलिंग विभिन्न कार्यों जैसे ग्लूकोज होमियोस्टेसिस, मेमोरी फॉर्मेशन, अनुभूति, ऊर्जा व्यय आदि को नियंत्रित करता है। दोषपूर्ण इंसुलिन सिग्नलिंग से मस्तिष्क में इंसुलिन प्रतिरोध होता है जो अल्जाइमर रोग (एडी), पार्किंसंस रोग (पीडी), हंटिंगटन रोग (एचडी) जैसे न्यूरोडीजेनेरेटिव रोगों के जोखिम को बढ़ाता है।

इंसुलिन सिग्नलिंग फॉस्फोराइलेशन / डिफॉस्फोराइलेशन संचालित कैस्केड है और काइनेज और फॉस्फेटेस की संतुलित क्रियाओं द्वारा नियंत्रित होता है। पिछले वर्षों में न्यूरोनल इंसुलिन सिग्नलिंग और इंसुलिन प्रतिरोध में कुछ काइनेज की भूमिका और भागीदारी को स्पष्ट करने का प्रयास किया गया है। हालाँकि, फॉस्फेटेस पर हमेशा कम ध्यान दिया जाता था। अमीनो एसिड अवशेषों के आधार पर वे डीफॉस्फोराइलेट करते हैं, फॉस्फेटेस को मोटे तौर पर तीन वर्गों में विभाजित किया गया था- टायरोसिन फॉस्फेटेस, सेरीन / थ्रियोनाइन फॉस्फेटेस और दोहरी विशिष्टता फॉस्फेटेस। सेरीन / थ्रियोनाइन फॉस्फेटेस आगे फॉस्फोप्रोटीन फॉस्फेटेस (PPP) परिवार में उप-विभाजित हैं, जिसमें PP1, PP2A और PP2B शामिल हैं, और प्रोटीन फॉस्फेटेस Mg^{2+} या Mn^{2+} निर्भर (PPM) परिवार, जिसमें PP2Cs शामिल हैं। जिनमें से PTEN (टायरोसिन फॉस्फेटेस) और SHIP2 (दोहरी विशिष्टता फॉस्फेटेस) को पहले न्यूरोनल इंसुलिन सिग्नलिंग के महत्वपूर्ण नियामकों के रूप में रिपोर्ट किया गया है। हालाँकि, न्यूरोनल इंसुलिन सिग्नलिंग और इंसुलिन प्रतिरोध में सेरीन / थ्रियोनाइन फॉस्फेटेस की भूमिका का निर्धारण करने वाली कोई रिपोर्ट नहीं है। इसलिए, वर्तमान अध्ययन में हमने निर्धारित किया (1) न्यूरोनल इंसुलिन सिग्नलिंग और इंसुलिन प्रतिरोध को विनियमित करने में PP2C α (PP2Cs के आइसोफॉर्म में से एक) की भूमिका, और (2) PP1 γ की भूमिका (PP1 के उत्प्रेरक सबयूनिट के आइसोफॉर्म में से एक) न्यूरोनल इंसुलिन सिग्नलिंग और इंसुलिन प्रतिरोध को विनियमित करने में।

Chapter 1 provides general introduction about insulin, insulin signaling and insulin resistance. It provides introduction about involvement of Ser/Thr phosphatases in insulin signaling. This chapter also discussed insulin signaling in brain and role of Ser/Thr phosphatases in neuronal insulin signaling and insulin resistance.

Chapter 2 describes the role of PP2C α in regulating neuronal insulin signaling and insulin resistance in addition to shedding light on the molecular mechanism involved.

Chapter 3 describes the role of PP1 γ in regulating neuronal insulin signaling and insulin resistance in addition to shedding light on regulating AD-like phenotypes.

Chapter 4 summarizes the present study, proposing its significance and possible future implications.

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ABBREVIATIONS

μM	Micromolar
2-NBDG	(2-(N-(7-Nitrobenz-2oxa-1,3-diazol-4-yl)-)amino)-2-deoxyglucose)
AD	Alzheimer's disease
AEBSF	4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride
AMPK	AMP-activated protein kinase
APP	Amyloid precursor protein
ARHGEF11	Rho guanine nucleotide exchange factor 11
AS160	AKT substrate of 160 kDa
AU	Arbitrary unit
BAT	Brown adipose tissue
BCA	Bicinchoninic acid
BORG4	Binder of Rho GTPase 4
CDK5	Cyclin dependent kinase 5
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
ERK	Extracellular signal-regulated kinase
FAK	Focal adhesion kinase
FBS	Fetal bovine serum
FOXO	Forkhead box transcription factors
GLUT4	Glucose transporter 4
GS	Glycogen synthase
GSK3	Glycogen synthase kinase 3
HD	Huntington's disease
HEPES	(4-(2-hydroxyethyl)-1piperazineethanesulfonic acid)
HFD	High-fat-fed diet
HNMPA-(AM)3	Hydroxy-2-naphthalenylmethylphosphonic acid triacetoxymethyl ester

IB	Immunoblot
IGFR	Insulin growth factor receptor
IP	Immunoprecipitation
IR	Insulin receptor
IRS	Insulin receptor substrate
JNK	c-Jun N-terminal kinase
kDa	Kilodalton
LTP	Long-term potentiation
MAPK	Mitogen activated protein kinase
MEM	Minimal essential media
MF	MCDB 201 and Ham's F-12 medium (1:1)
MFI	MCDB 201 and Ham's F-12 medium (1:1) +100 nM insulin
MLK3	Mixed-lineage protein kinase 3
mM	Millimolar
MRIP	Myosin phosphatase Rho-interacting protein
ND	Normal diet
NFT	Neurofibrillary tangles
nM	Nanomolar
OA	Okadaic acid
PAGE	Polyacrylamide gel electrophoresis
PAK	p21-activated kinase
PBS	Phosphate-buffered saline
PD	Parkinson's disease
PHLPP	PH domain and leucine rich repeat protein phosphatases
PI3K	Phosphoinositide 3-kinase
PKC	Protein kinase C
PMSF	Phenylmethanesulfonyl fluoride
PP1R12A	Protein phosphatase 1 regulatory subunit 12 alpha

PP2Ac	Phosphoprotein phosphatase 2 alpha catalytic subunit
PPM	Metal-dependent protein phosphatase
PPP	Phosphoprotein phosphatase
PPP	Picropodophyllin
PPP1R12A	Protein phosphatase 1 regulatory subunit 12A
PPP2C α	Phosphoprotein phosphatase 2 catalytic subunit alpha
PPP2R5D	Phosphoprotein phosphatase 2 regulatory subunit 5 delta
PTEN	Phosphatase and TENsin homolog
PTP	Protein tyrosine phosphatase
RT-PCR	Real-time polymerase chain reaction
SC	Sanguinarine chloride
SDS	Sodium dodecyl sulfate
SE	Standard error
SGOT	Serum glutamic-oxaloacetic transaminase
SHP2	Src homology region 2 domain-containing phosphatase-1
siRNA	Small interfering RNA
SNRK	Sucrose non-fermenting 1-related kinase
SPGT	Serum glutamic-pyruvic transaminase
STZ	Streptozotocin
T2D	Type 2 diabetes
TBST	Tris-buffered saline tween-20
TEMED	N,N,N',N'-Tetramethylethylenediamine
ThS	Thioflavin S
WAT	White adipose tissue