SER/THR PHOSPHATASES PP1γ AND PP2Cα IN REGULATING NEURONAL INSULIN SIGNALING AND INSULIN RESISTANCE

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DECEMBER 2022

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(2016BLZ8134)

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Submitted

In fulfillment of the requirements of the degree of

Doctor of Philosophy

to the

Indian Institute of Technology Delhi

December 2022

Dedicated to
my mother
and
all my teachers

Certificate

This is to certify that the thesis entitled **"Ser/Thr phosphatases PP1γ and PP2Cα 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
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Date: 28th December 2022
Place: New Delhi

Acknowledgements

I take this opportunity to express my sincere gratitude to my Ph.D. mentor **Prof. Chinmoy Sankar Dey** for his timely guidance and constant motivation throughout the course of my Ph.D research. I thank him for the contribution of his valuable time and funding that helped to carry out my research work smoothly. Sir's research aptitude, enthusiasm and the scientific discussion with him made my Ph.D research a great learning experience. Being one of his Ph.D. students at IIT Delhi, I got an opportunity to learn what is required to establish a functional laboratory. He encouraged me to pursue my ideas and plan my experiments independently, in a disciplined manner that greatly helped me to grow as a researcher. I am extremely thankful to my student research committee (SRC) members: **Prof. James Gomes, Prof. Vivekanandan Perumal, Prof. D. Sundar** and **Prof. Biren Mullick** (Jawaharlal Nehru University, Delhi, India) for providing me valuable suggestions that helped me improve my research. I would like to acknowledge the faculty members of Kusuma School of Biological Sciences: **Prof. B Jayaram, Prof. Aditya Mittal, Prof. Amitabha Mukopadhyay, Prof. Tapan Kumar Chaudhary, Prof. Bishwajit Kundu, Dr. Archana Chugh, Dr. Ashok Kumar Patel, Dr. Anita Roy, Dr. Manoj Menon, Dr. Saran Kumar, Dr. Saurabh Raj, Dr. Shilpi Minocha, Dr. Tapan K Nayak, Dr. Vivekanandan Perumal, Dr. Manidipa Banerjee** for providing the research facilities necessary to conduct the experiments. My special thanks to **Dr. Prosenjit Mondal**, Indian Institute of Technology-Mandi, Himachal Pradesh, India, for providing us with normal diet (ND) and high fat fed diet (HFD) mouse brain samples; **Prof. Ellora Sen**, National Brain Research Centre, Delhi for providing us pJNK and JNK antibodies. We are grateful to Central Research Facility, Sonipat Campus, Indian Institute of Technology-Delhi, Haryana, India for Confocal Microscopy. The work done in this thesis would not have been possible without the facility of fluorescence spectrophotometer that has been kindly provided by **Prof. Bishwajit Kundu**. I would like to thank their students **Ms. Anjali Dixit** for her

kind cooperation in helping me to use this facility. I would like to thank **Council of Scientific and Industrial Research, Government of India**, New Delhi, India for the research grant to Prof. Dey (grant number: [27(0346)/19-EMR-ii]). I want to thank **Department of Science and Technology (DST)**, for providing me Junior Research Fellowship and Senior Research Fellowship throughout the tenure of my PhD research. I acknowledge **Kusuma trust** for funding the department. Furthermore, I would like to acknowledge the support of my senior lab members: **Dr. Abhijeet Chowdhary, Dr. Pallavi Varshney, Dr. Amita Arora, Dr. Kapil Manglani and Dr. Aakash Mukopadhyay** for training me with lab techniques to pursue my objectives. I would also want to thank my lab members **Ms. Medha Sharma, Ms. Devanshi Mishra, Dr. Shankari Prasad Dutta, Ms. Ishitha Reddy** and **Mr. Ayan Mallik** for providing a great work environment in the laboratory. My special thanks to **Ms. Medha Sharma** for her constant support. Besides, I appreciate her jovial attitude and I always enjoyed her company. We shared many moments that will always be remembered and cherished. I would like to thank **Dr. Archana Chugh** for providing me the opportunity to use fluorescence microscope and her Ph.D. students **Ms. Anjali Priya** and **Ms. Prasanjit Kaur** for helping me capturing the images at fluorescence microscope. I gratefully acknowledge the support provided by my senior **Dr. Nitika Gaurav** and friends **Ms. Firdos Farooq** and **Ms. Anjali Dixit** throughout the tenure of my Ph. D research. I thank them for patiently hearing all my joys and difficulties. I want to thank the staff members of Kusuma School of Biological Sciences: **Ms. Mini** and **Mr. Pradeep Tripathi** for helping in administrative processes. My heartfelt gratitude to **my mother** for love, care and support which cannot be described in a few words. I thank them for standing by my side in my difficult times. Took care of everything that I required so that I could focus completely on my Ph.D. work. Last but not the least

I thank the **Lord Almighty** for blessing me with amazing family and friends. I thank God for providing me the opportunity and strength to accomplish my Ph.D. degree.

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 Mg2+ or Mn2+ -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 PP2Cα (one of the isoforms of PP2Cs) in regulating neuronal insulin signaling and insulin resistance, and (2) the role of PP1γ (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। हालांकि, 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 / थ्रेओनाइन फॉस्फेटेस और दोहरी विशिष्टता फॉस्फेटेस। सेरीन / थ्रेओनाइन 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। हालांकि, न्यूरोनल इंसुलिन सिग्नलिंग और इंसुलिन प्रतिरोध में सेरीन / थ्रेओनाइन 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 PP2Cα (PP2Cs के आइसोफॉर्म में से एक) की भूमिका, और (2) PP1γ 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 PP2Cα in regulating neuronal insulin signaling and insulin resistance in addition to shedding light on the molecular mechanism involved.

Chapter 3 describes the role of PP1γ 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
PPP2Cα	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