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

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Insulin action is reported important for the brain. It regulates energy homeostasis, eating behavior, cognitive tasks etc. Disruption of insulin action in brain leads to a neuronal insulin resistance causing impairment of neuronal functions. In addition, insulin signaling regulates the phosphorylation of Tau protein, a protein involved in progression of AD pathogenesis. Thus, alterations in insulin action in brain leads to metabolic disorders and neurodegenerative diseases. Altogether it is clear that insulin signaling is important for brain yet information regarding neuronal insulin signaling and insulin resistance is still very limited. Insulin signaling is under tight regulation of phosphorylation; where kinases and phosphatases holds equal importance. Despite being important for insulin signaling, the role of Ser/Thr phosphatases in regulating neuronal insulin signaling still largely remained unanswered. In the past, few evidences focused on the participation of Ser/Thr phosphatases in insulin signaling in peripheral tissues like skeletal muscle, adipocytes, hepatocytes etc. Few preliminary studies have highlighted the role of isoform of one of the Ser/Thr phosphatase as well as isoforms of their catalytic and regulatory subunits in insulin signaling. These studies were carried out in other insulin signaling peripheral tissues, however, their role in neuronal insulin signaling is completely unexplored. Information about various catalytic and regulatory subunits of Ser/Thr phosphatases in peripheral tissues provided the idea that the substrate specificity of any phosphatase lies in the binding of their different catalytic and regulatory subunits. However, detailed studies are required in order to deeply understand the role and importance of phosphatases and their catalytic and regulatory subunit in regulating neuronal insulin signaling.

Therefore, in present study attempts were made in order to determine (1) The role of one of the isoforms of PP2C family of Ser/Thr phosphatase i.e., PP2Cα. (2) The role of catalytic subunit of PP1 family of Ser/Thr phosphatase i.e., PP1γ in neuronal insulin signaling and insulin resistance.

To fulfill the above-mentioned objectives mammalian neuroblastoma cell lines of two different species i.e., mouse (N2a) and human (SH-SY5Y) were utilized. For determining the role of PP2Cα and PP1γ in neuronal insulin resistance, *in-vitro* model of neuronal insulin resistance was employed. This model was previously generated in our laboratory wherein mouse (N2a) and human (SH-SY5Y) cells were differentiated for 3 days and 4 days respectively in absence (insulin sensitive) or chronic presence (insulin resistant) of insulin. In order to determine the role of PP2Cα and PP1γ in mice whole brain in insulin resistant condition, we utilized the whole mice brain lysates of High-fat-diet (HFD) fed insulin resistant mice and Normal-diet (ND) fed mice (kind gift from Dr. Prosenjit Mondal, Indian Institute of Technology-Mandi). To elucidate the role of PP2Cα and PP1γ in neuronal insulin signaling and insulin resistance we first determined their respective expressions in N2a and SH-SY5Y cells as well as mice whole brain lysates. Further the participation of these phosphatases in regulating neuronal insulin signaling was determined by either inhibition (by specific inhibitors) or downregulation (by specific siRNA). The key findings of this study are as follows:

1. Insulin rapidly increased the expression of PP2Cα under insulin sensitive condition. This expression was reduced under insulin resistant condition.
2. Inhibition studies revealed that this rapid increase in the expression of PP2Cα occurred at the level of translation and not at transcription.
3. Inhibiting various signaling pathways demonstrated the involvement of MAPKs among which JNK is the kinase regulating the expression of PP2Cα.
4. Interestingly it was found that, under insulin sensitive condition, rapid activation of JNK was sufficient to increase the expression of PP2Cα in response to insulin.
5. Similar to insulin sensitive condition, reduction in expression of PP2Cα under insulin resistance is regulated at translation level through JNK.

6. Inhibition and downregulation of PP2Cα provided the evidences of positive role of PP2Cα in neuronal insulin signaling and insulin resistance.
7. PP2Cα acts on a negative regulator i.e., AMPK and inhibitory phosphorylation of IRS-1 (Ser522) and promotes neuronal insulin signaling.
8. On the other hand, both catalytic subunits of PP1 i.e., PP1α and PP1γ are expressed in neurons.
9. On contrary to PP2Cα, the expression of PP1α and PP1γ are unaltered in response to insulin both under insulin sensitive and insulin resistant condition.
10. Downregulation of both PP1α and PP1γ revealed that PP1γ and not PP1α is involved in regulating neuronal insulin signaling and insulin resistance.
11. Among three isoforms of AKT i.e., AKT1, AKT2 and AKT3, PP1γ regulates the dephosphorylation of AKT2 which in turn regulates neuronal glucose uptake.
12. PP1γ also regulates the dephosphorylation of GSK3 isoforms (GSK3α and GSK3β) in an opposite manner.
13. PP1γ regulates the phosphorylation of GSK3β by regulating the phosphorylation of AKT2, while it regulates the phosphorylation of GSK3α by regulating the phosphorylation of MLK3.
14. Due to PP1γ effects on GSK3 isoforms, PP1γ fine tunes AD-like phenotypes in neuronal system. On one hand the silencing of PP1γ increases the formation of Aβ plaques while on the other hand silencing of PP1γ decreases the formation of NFTs.

Overall, the data thereby reports the role and mechanism of action of PP2Cα and PP1γ in regulating neuronal insulin signaling and insulin resistance. Elucidating the role isoforms of phosphatases and isoforms of catalytic subunits of phosphatases in neuronal insulin signaling will be beneficial to deeply understand how a single phosphatase is able to act on two or more different kinases. Studies to discover the contribution of Ser/Thr phosphatases in regulating

neuronal insulin signaling and insulin resistance might connect metabolic syndrome like AD. In future it will be motivating to dig deeper highlighting the role of catalytic/regulatory subunits of Ser/Thr phosphatases, which may provide novel link between disrupting Ser/Thr phosphatases and treating diseases like Type 3 diabetes.