In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (not to exceed 3000 words)

1.Sharma M and **Dey CS**: Role of Akt isoforms in neuronal insulin signaling and -resistance. **Cell. Mol. Life Sci.** 2021, 78, 7873. **IF: 9.207**.

Our study is the first to report isoform specific role of all Akt isoforms in regulating neuronal insulin-signaling and -resistance. By silencing Akt isoforms individually and in-pairs, in Neuro-2a and HT22 cells we observed that, in insulin-sensitive condition, Akt isoforms differentially reduced activation of AS160 and glucose uptake with Akt2 playing the major role. We find that in neuronal cells (a) all Akt isoforms regulate AS160 activation and glucose uptake; Akt2 play a predominant role, with Akt1 and Akt3 playing significant role as well; (b) Activation of all isoforms decreased differentially under insulin-resistance with Akt2 being affected most, followed by Akt3 and Akt1; (c) Insulin-resistance is reversed by over-expression of any isoform of Akt, but predominantly by Akt2; (d) Insulin-dependent translocation on plasma membrane determines isoform specificity with Akt2 translocating the most, followed by Akt3 and then Akt1; (e) Insulin-resistance hampered this insulin-dependent translocation of all Akt isoforms to plasma membrane, irrespective of isoform; (f) Akt3, despite being neuron specific isoform contributed substantially to AS160 regulation, neuronal glucose uptake, and insulin-resistance. However, Akt2 was still the predominant isoform in regulating all the above functions. This points to a novel, differential yet compensatory interplay of all Akt isoforms in neuronal insulin signaling and insulin-resistance. These findings are fundamentally important on their own right for deeper understanding of insulin signaling and insulin-resistance in neuronal cells. This may in future help in solving a spectrum of problems associated with a diabetes, diabetes complications and neurodegenerative disorders.

2. Gupta, A; Bisht, B and **Dey CS**: Peripheral insulin-sensitizer drug Metformin ameliorates neuronal insulin resistance and Alzheimer's-like changes. **Neuropharmacology** 2011, 60:910. **IF: 5.273**.

Alzheimer’s disease (AD) is the most common neurodegenerative disease worldwide. Pharmacological treatments presently available can slow down the progression of symptoms but cannot cure the disease. Currently there is widening recognition that AD is closely associated with impaired insulin signaling and glucose metabolism in brain, suggesting it to be a brain-specific form of diabetes and so also termed as “type 3 diabetes”. Hence investigating the role of pharmacological agents that could ameliorate neuronal insulin resistance merit attention in AD therapeutics, however the therapeutics for pathophysiological condition like neuronal insulin resistance itself is largely unknown. The present study determined the effect of metformin on neuronal insulin resistance and AD-associated characteristics in an *in vitro* model of “type 3 diabetes” by differentiating neuronal cell line Neuro-2a under prolonged presence of insulin. Prolonged hyperinsulinemic conditions in addition to generating insulin resistance also led to development of hallmark AD-associated neuropathological changes. Treatment with metformin sensitized the impaired insulin actions and also prevented appearance of molecular and pathological characteristics observed in AD. The results thus demonstrate possible therapeutic efficacy of peripheral insulin sensitizer drug metformin in AD by its ability to sensitize neuronal insulin resistance. These findings also provide direct evidences linking hyperinsulinemia and AD and suggest a unique opportunity for prevention and treatment of “type 3 diabetes”.

3. Yadav Y, Sharma M and **Dey CS**: PP1γ regulates neuronal insulin signaling and aggravates insulin resistance leading to AD-like phenotypes. **Cell Commun.Signal.** 2023, 21, 82. **Impact Factor: 7.525**.

PP1γ is one of the isoforms of catalytic subunit of a Ser/Thr phosphatase PP1. The role of PP1γ in cellular regulation is largely unknown. The present study investigated the role of PP1γ in regulating neuronal insulin signaling and insulin resistance in neuronal cells. The expression of PP1α and PP1γ was determined in insulin resistant N2a, SH-SY5Y cells and in high-fat-diet-fed-diabetic mice whole-brain-lysates and they were silenced by siRNA and effect was tested on AKT isoforms, AS160 and GSK3 isoforms, GLUT4 translocation and glucose uptake. Results showed that, in one hand PP1γ, and not PP1α, regulates neuronal insulin signaling and insulin resistance by regulating phosphorylation of AKT2 via AKT2-AS160-GLUT4 axis. On the other hand, PP1γ regulates phosphorylation of GSK3β via AKT2 while phosphorylation of GSK3α via MLK3. Imbalance in this regulation results into AD-like phenotype. Utilizing two different cell lines and partially utilizing animal models the study determined that PP1γ regulates neuronal insulin signaling and insulin resistance by dephosphorylating AKT2 and is also involved in regulating AD-like phenotypes through GSK3. Therefore, the study shows first ever evidences that PP1γ acts as a linker, regulating two pathophysiological conditions, neuronal insulin resistance and AD.

4.Sharma M and **Dey CS**: PHLPP isoforms differentially regulate Akt isoforms and AS160 affecting neuronal insulin signaling and insulin resistance via Scribble. **Cell Commun. Signal.** 2022, 20, 179. **IF: 7.525**.

Our study reports isoform specific role of both PHLPP isoforms in regulating neuronal insulin signaling and resistance. We find the following: (a) elevated expression of both PHLPP1 and PHLPP2 in insulin resistant neuronal cells and high-fat-diet fed mice whole brain lysate.; (b) PHLPP1 regulates serine phosphorylation of Akt2 and Akt3, and PHLPP2 regulates serine phosphorylation of Akt1 and Akt3 in neuronal cell insulin signaling; (c) PHLPP1 regulates serine phosphorylation of Akt2 and Akt3, and PHLPP2 regulates serine phosphorylation of Akt1 and Akt3 in insulin resistant neuronal cells; d) both PHLPP isoforms regulate three Akt isoforms, extending the regulation to AS160 (e) both the isoforms of PHLPP regulate glucose uptake in insulin sensitive and insulin resistant neuronal cells (f) PHLPP isoform specificity is mediated by Scribble, which mediates cellular localization of isoforms, regulating neuronal insulin signaling; (g) a novel role of Scribble in regulating glucose uptake in neuronal cells. All data points to a differential and independent role of PHLPP isoforms in neuronal insulin signaling and insulin resistance. These observations are significant and insightful in understanding insulin signaling and insulin resistance. Further, this study in neuronal insulin resistance, which is one of the hallmarks of several neurodegenerative diseases, may help in taking a step forward in solving problems associated with a Type 3 diabetes, diabetes complications and neurodegenerative disorders.

5.Yadav Y and **Dey CS**: PP2Cα positively regulates neuronal insulin signaling and aggravates neuronal insulin resistance being translated by insulin. **FEBS J.** 2022, 289, 7561. **IF: 5.622**.

PP2Cα is amongst one of the new member isoforms of metal-dependent protein phosphatases (PPM). The role of this phosphatase in neuronal insulin signaling is completely unknown. We for the first time are reporting insulin mediated rapid upregulation of a protein

of insulin signaling cascade, PP2Cα. In contrast, this expression does not happen in insulin resistant condition despite insulin stimulation. Overall, the study suggests that under normal conditions when PP2Cα is active, in presence of insulin, PP2Cα keep IRS-1 dephosphorylated at Ser522, promoting neuronal glucose uptake through PI3K-AKT-AS160 pathway. When PP2Cα is diminished, either by its inhibition or by knockdown, it is unable to dephosphorylate IRS-1 at Ser522, causing reduction in activation of kinases, like AKT, followed by AS160, hence reducing the glucose uptake. Through this study, we for the first time have added a new member in the list of insulin mediated rapid expression of a phosphatase, PP2Cα via JNK, in neuronal insulin signaling as well as in insulin resistance. We explored the mechanism of enhanced expression of PP2Cα under insulin sensitive condition and reduced expression under insulin resistance by elucidating the positive role of PP2Cα in regulating neuronal insulin signaling and insulin resistance. This signifies that PP2Cα could be a new possible candidate to target and manage type 2 and type 3 diabetes in future.

6.Yadav Y and **Dey CS**: PP2Cα aggravates neuronal insulin resistance leading to AD-like phenotype *in vitro*. **Biochem. Biophys. Res. Commun.** 2023, 644, 49. IF: 3.322

The α isoform of Protein Phosphatase-2C (PP2C) is a Ser/Thr phosphatase, only known in 3T3-L1 adipocytes as a positive regulator of insulin signaling. However, many aspects of its function in neuronal insulin signaling and insulin resistance are unidentified. We studied the role of PP2Cα in regulating activities of both isoforms of GSK3α and GSK3β (one of the leading kinases for AD progression). Silencing of PP2Cα caused hyperphosphorylation of a potential kinase Tau, leading to NFT formation and increased Aβ deposition. Our study thereby demonstrates escalation of hyperinsulinemia mediated neuronal insulin resistance leading to AD-like pathogenesis by PP2Cα.

7.Bisht B, Srinivasan K and **Dey CS.** *In vivo* inhibition of Focal Adhesion Kinase causes insulin resistance. **J. Physiology,** 2008, 586/16: 3825**. IF: 6.228**.

Focal adhesion kinase (FAK) had been implicated in the regulation of insulin resistance in vitro previously from the laboratory. However, its in vivo validation has not been attempted due to lethality of FAK knockout. Hence, to ascertain the role of FAK in the development of insulin resistance in vivo, FAK expression was down-regulated by delivering FAK-specific small interfering RNA (siRNA) in mice using hydrodynamic tail vein injection. FAK silencing exacerbates insulin signalling and causes hyperglycaemia and hyperinsulinaemia in vivo. FAK-silenced animals were less glucose tolerant and had physiological and biochemical parameters similar to that of high fat diet (HFD)-fed insulin-resistant animals. Phosphorylation and expression of insulin receptor substrate 1 (IRS-1) was attenuated in muscle and in liver in FAK-silenced mice. Akt-Ser473-phosphorylation decreased in muscle and liver in FAK-silenced mice. This, in part, explains the mechanism of development of insulin resistance in FAK-silenced mice. The present study provides direct evidence that FAK is a crucial mediator of insulin resistance in vivo. Considering the lethality of FAK gene knockout the approach of this study will provide a new strategy for in vivo inhibition of FAK. Furthermore, the study should certainly motivate chemists to synthesize new chemical entities for FAK activation. This may shed light on new drug development against insulin resistance.

8.Gupta A, Bisht B, **Dey CS**: Focal adhesion kinase negatively regulates neuronal insulin resistance. **Biochim Biophys Acta.-Molecular Basis of Disease**.2012:1822:1030. **IF: 6.63**.

Focal adhesion kinase (FAK), a non-receptor protein kinase, is known to be a phosphatidyl inositol 3-kinase (PI3K) pathway activator and thus widely implicated in regulation of cell survival and cancer. FAK has also been strongly implicated as a crucial regulator of insulin resistance in peripheral tissues like skeletal muscle and liver, where decrease in its expression/activity has been shown to lead to insulin resistance. However, the present study report an altogether different role of FAK in regulation of insulin/PI3K signaling in neurons, the post-mitotic cells. An aberrant increase in FAK tyrosine phosphorylation was observed in insulin resistant N2A cells. Downregulation of FAK expression utilizing RNAi mediated gene silencing in insulin resistant N2A cells completely ameliorated the impaired insulin/PI3K signaling and glucose uptake. FAK silencing in primary cortical neurons also showed marked enhancement in glucose uptake. The results thus suggest that in neurons FAK acts as a negative regulator of insulin/PI3K signaling. Interestingly, the available literature also demonstrates cell-type specific functions of FAK in neurons. FAK that is well known for its cell survival effects has been shown to be involved in neurodegeneration. Present findings highlight a novel and critical role of FAK in neurons. Moreover, as this implicates differential regulation of insulin/PI3K pathway by FAK in peripheral tissues and neuronal cells, it strongly suggests precaution while considering FAK modulators as possible therapeutics.

9.Varshney P and **Dey CS:** P21-activated kinase 2 (PAK2) regulates glucose uptake and insulin sensitivity in neuronal cells. **Mol. Cell. Endocrinol.** 2016, 429, 50-61**.** IF: 4.4

P21-activated kinases (PAKs) are recently reported as important players of insulin signaling and glucose homeostasis in tissues like muscle, pancreas and liver. However, their role in neuronal insulin signaling is still unknown. Present study reports the involvement of PAK2 in neuronal insulin signaling, glucose uptake and insulin resistance. Irrespective of insulin sensitivity, insulin stimulation decreased PAK2 activity. PAK2 down regulation displayed marked enhancement of glucose uptake whereas PAK2 over-expression showed reduction. Results with inhibitors like Akti-1/2 and wortmannin suggested both Akt and PI3K as mediators of insulin effect on PAK2 and glucose uptake. Rac1 inhibition demonstrated decreased PAK2 activity while inhibition of phosphatase PP2A resulted in an increase in PAK2 activity, with corresponding changes in the glucose uptake. Taken together, present study demarcates the inhibitory role of insulin signaling (via PI3K-Akt) and PP2A on PAK2 activity and establishes PAK2 as a Rac1-dependent negative regulator of neuronal glucose uptake and insulin sensitivity.

10.Gupta A and **Dey CS**: PTEN, a widely known negative regulator of insulin/PI3K signaling, positively regulates neuronal insulin resistance. **Mol. Biol. Cell** 2012 23(19):3882-98. IF: 6.0

Lipid and protein tyrosine phosphatase, PTEN, is a widely known negative regulator of insulin/PI3K signaling. Down regulation of PTEN is thus widely documented to ameliorate insulin resistance in peripheral tissues like skeletal muscle and adipose. However, not much is known about its exact role in neuronal insulin signaling and insulin resistance. Moreover, alterations of PTEN in neuronal systems has led to discovery of several unexpected outcomes including in neurodegenerative disorder, Alzheimer's disease (AD), which is increasingly being recognised as a brain-specific form of diabetes. Present study reports that PTEN,

paradoxically, positively regulates neuronal insulin signaling and glucose uptake. Its downregulation exacerbates neuronal insulin resistance. The positive role of PTEN in neuronal insulin signaling is likely due to its protein phosphatase actions, which prevents the activation of FAK and ERK, the kinases critically involved in neuronal energy impairment and neurodegeneration. Results suggest that PTEN acting through FAK, the direct protein-substrate of PTEN, prevents ERK activation. These findings provide explanation for unexpected outcomes earlier reported with PTEN alterations in neuronal systems and also propose a novel molecular pathway linking neuronal insulin resistance and AD, the two pathophysiological states that are being demonstrated to be closely interlinked.