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GAVIN PUBLISHERS

Research Advances in Brain Disorders and Therapy

Short Communication

Pant HC. Res Adv Brain Disord Ther RABDT-101.

DOI: 10.29011/ RABDT-101. 100001

A Novel Approach to Ameliorate and Provide a Protective Role in Neurodegenerative Diseases by A Novel Peptide, TFP5/TP5, Derived from Cdk5 Activator P35

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Citation: Pant HC (2018) A Novel Approach to Ameliorate and Provide a Protective Role in Neurodegenerative Diseases by A Novel Peptide, TFP5/TP5, Derived from Cdk5 Activator P35. Res Adv Brain Disord Ther RABDT-101. DOI: 10.29011/ RABDT-101. 100001

Received Date: 14 March, 2018; **Accepted Date:** 27 March, 2018; **Published Date:** 2 April, 2018

Communication

The Etiology of AD and other age-related neurodegenerative disorders is complex, involving as it does, many multivariate interacting pathways among which are aging itself, inflammation, mitochondrial ROS disorders, hyperactive kinases such as Cdk5, mutations in Amyloid Precursor Protein (APP), secretases and tau processing. The identification of gene mutations responsible for neurodegeneration in humans has led to development of a variety of transgenic mouse models, each expressing a neurodegenerative phenotype such as AD, PD or ALS. For AD, for example, an extensive model mouse literature has accumulated validating one or another pathway as specifically responsible for the behavioral and pathological disease phenotype (e.g. Elder et al, 2010, Landreth et al, 2012). And, in some instances, these mutant mice have been claimed successfully "cured" or "rescued" to some extent by agents or manipulations that restore the damaged pathways. These are touted as potential therapeutic candidates.

Our current research program evolved after years of basic science studies of neuronal cytoskeletal protein phosphorylation during nervous system development and function, a brief summary of our accomplishments over the years should illustrate how we arrived at our present program. We found that neurofilaments, the major axonal proteins, are selectively phosphorylated in axons [1-4]. Using a neurofilament assay, our laboratory identified Cyclin Dependent Kinase 5 (Cdk5), together with its activator, P35, as one of the principal kinases regulating neuronal topographic phosphorylation biology and physiology. The multifunctional kinase, Cdk5, was initially characterized as a tau protein kinase, a proline directed kinase [5] or a neuronal cdc-2 like kinase in our lab**,** presently known as Cdk5**.** We as well as other laboratories have shown that Cdk5 is a tightly regulated multifunctional kinase essential for neuronal development, neurogenesis, migration, synaptic activity, memory / learning and survival, phosphorylating a large number of target protein substrates [6-11].

Cdk5 when deregulated by neuronal stress (e.g., glutamate excitotoxicity, Aβ toxicity, Reactive Oxygen Species, ROS, and others), Cdk5 activity is deregulated and hyperactivated as a stable complex with p25 (a truncated fragment of p35, a major activator of Cdk5) and induces Perikaryal hyper phosphorylated tau, Neuro filament Proteins (NF-M/H), and other neuronal intermediate filament proteins as seen in AD, PD and ALS [12,13], thus Cdk5/p25 becomes a pathological target. This relationship to AD and other pathologies has been documented in studies of AD brains showing high levels of p25, reduced p35/p25 ratios and Cdk5 hyperactivity [14,15], Furthermore, we have confirmed that Cdk5/p25 induces tau and NFP aberrant hyperphosphorylation along with cell death in cultured cortical neurons [15,16]. Consistent with this hypothesis is a p25-overexpressing model mouse, developed by Dr. Tsai's lab and recently our own lab, that displays the typical AD Abnormal Phenotype [17]. Accordingly, hyperactive Cdk5/p25 has been identified as a possible therapeutic target for neurodegeneration. Recently, a more compelling role of hyperactive Cdk5/p25 as a significant factor in the etiology of AD comes from studies of cell cultures *in vitro* [16] and model mice *in vivo* as documented in three publications [18,19 and 20]. Since the binding of p25, the proteolytic fragment of p35, induces deregulated and hyperactivated Cdk5, we asked the question what is the role of smaller truncated peptides of p25 in the regulation of

Cdk5 activity? This has led us to the isolation and identification of peptides derived from p35 (CIP , cdk5 inhibitory peptide and P5, a 24 amino acid peptide derived from p25) that specifically inhibited the hyperactive Cdk5/p25 without affecting the physiologically normal Cdk5/p35 [16] Consistent with the model, we succeeded in showing that pathological and behavioral phenotypes in AD model mice (over-expressing p25 transgenic) and the 5XFAD transgenic can be alleviated after treatment with CIP and TFP5, our *in vivo* therapeutic reagents.

We viewed these peptides as potential therapeutic candidates for rescuing neurodegenerative disorders in model mice sharing the hyperactivated Cdk5-induced phenotypes. Currently, most therapeutic approaches targeting the deregulated Cdk5/p25 complex and other kinases in neurodegenerative disorders and cancer have focused primarily on drugs like roscovitine, ATP analog, that inhibit Cdk5 activity by interfering with the ATP binding domain of the kinase. Most of these drugs, however, lack sufficient specificity, since all kinases including cell cycle Cdks including Cdk5, are vulnerable at the ATP binding site targeted by these drugs. In order to make P5, small 24 amino acid peptides an *in vivo* therapeutic reagent, we coupled the C-terminus of P5 to a Protein Transduction Domain Peptide (PTD -TAT) and its N-terminus to FITC, Fluorescent Tag, Fluorescein Isothiocynate. This reagent that we call TFP5 was shown to pass the blood brain barrier and to rescue the AD phenotype in AD model mice. Hence, next, we conducted the following studies:

Effects of TFP5 on expression of AD phenotypes in a p25Tg over-expressing and double transgenic (5XFADTg) AD model mice, mechanisms of specificity of TFP5 action and effect of TFP5 on ALS model mice (in preparation).

At the forefront of the AD literature are those model mice expressing the two landmark pathologies seen at human autopsy, hyper phosphorylated intracellular cytoskeletal tangles (tau, neurofilaments) and extracellular amyloid plaques. One of the many hypotheses invoked to explain these phenotypes is the role of deregulated, hyperactive Cdk5/p25, reported at elevated levels in AD brains at autopsy [14]. From these and other related studies *in vivo* and *in vitro*, a hypothesis has been proposed to account for the deregulation of Cdk5, and its induction of tau and amyloid pathology leading to the chronically long descent into the abyss of dementia. A most persuasive validation of the hypothesis is overexpression of p25 in a mouse model that induces Cdk5 hyperactivity, AD pathology, behavioral defects and early mortality [17]. Elevated levels of p25 in post-mortem AD brains, however, though supported by the above (and other reports), was not confirmed in a few laboratories. These differences had been, in part, attributed to tissue sampling conditions and preparation protocols. Nevertheless, the weight of evidence from our lab and others is consistent with the hypothesis; hyperactive Cdk5/p25 has been identified as a target in neurodegeneration and many compounds that inhibit this kinase have been tested in AD model mice [19]. Thus, these studies demonstrate first time, the peptide TFP5/TP5 ameliorates familial (5XFAD) and sporadic p25Tg phenotypes in both AD model mice.

Acknowledgements

Author will like to thank all the members of the laboratory and collaborators current and past for their help and support to accomplishment this work.

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