Glycolytic enzyme Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and Neurological Disorders

[1]Imran Ahmad, [2]Nidhi Sachan, [1]Shayan Mohd, [3]Arbab Husain

[1]Environmental Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow.

[2]Toxicogenomics and Predictive Toxicology Laboratory, Systems Toxicology and Health Risk Assessment GroupCSIR-Indian Institute of Toxicology Research, LucknowIndian.

[3]Department of Biosciences, Integral University, Lucknow

Corresponding Author: imranahmad008@gmail.com

Abstract

Glyceraldehyde 3-phosphate dehydrogenase *(abbreviated as* ***GAPDH*** *or less commonly as GAPDH) (EC 1.2.1.12) is an enzyme of ~37kDa that catalyses the sixth step of glycolysis and thus serves to break down glucose for energy and carbon molecules. GAPDH initially identified as a glycolytic enzyme and considered as a housekeeping gene, is widely used as an internal control in experiments on proteins, mRNA, and DNA. GAPDH is encoded by a single gene on human chromosome 12 that gives rise to an individual mRNA transcript with no known splice variants. GAPDH is tightly regulated at transcriptional and posttranscriptional levels, which are involved in the regulation of diverse GAPDH functions. Several factors such as insulin, hypoxia inducible factor-1 (HIF-1), p53, nitric oxide (NO), and acetylated histone, not only modulate GAPDH gene expression but also affect protein functions via common pathways. Oxidoreductase GAPDH has become a subject of interest as studied revealed a surfeit of diverse GAPDH functions depending on its post-translational modifications (PTMs), extending beyond traditional aerobic metabolism of glucose. As a result of multiple isoforms, cellular locales and diverse functions, GAPDH is able to come in contact with a variety of small molecules, proteins, membranes, etc., that play important roles in normal and pathologic cell function. Specially, GAPDH has been implicated in several neurodegenerative diseases and disorders, largely through interactions with other proteins specific to that disease or disorder. For example, GAPDH interactions with beta-amyloid precursor protein (beta APP) could interfere with its function regarding the cytoskeleton or membrane transport and can cause Alzheimer disease (AD), while interactions with Huntington could interfere with its function regarding apoptosis, nuclear tRNA transport, DNA replication, and DNA repair. In addition, nuclear translocation of GAPDH has been reported in Parkinson's disease (PD), and several anti-*

apoptotic PD drugs, such as rasagiline, function by preventing the nuclear translocation of GAPDH. In this review, recent findings related to GAPDH and Neurological disorder are summarized.

Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme that is responsible for the sixth step of glycolysis[1]. In addition to this metabolic function, GAPDH is now recognized as a multifunctional protein that exhibits other functions, including DNA repair[2], transcriptional and posttranscriptional gene regulation[3], intracellular membrane trafficking[4], and cell death[5,6]. In GAPDH mediated cell death pathway, the involvement of GAPDH in nuclear translocation and its aggregation under oxidative stress have been proposed[7–9]. The active-site cysteine (Cys-152) seems to play a crucial role in both pathways. For example, GAPDH binds to Siah (seven in absentia homolog) through oxidation/S-nitrosylation of Cys-152 and translocate into the nucleus in response to oxidative stress, such as that from nitric oxide(NO)[10]. Nuclear GAPDH activates p300/CREB (cAMP-response element-binding protein)-binding protein (CBP)[11]andpoly(ADP-ribose)polymerase-1[12]. Additionally, oxidative stress orsinitiate amyloid- like GAPDH aggregation via intermolecular disulphide bond sat Cys-152[13–15].

The accumulation of unfolded proteins can cause protein aggregation in the aged brain, and these aggregates facilitate the formation of pathological amyloid deposits, which is a key cause of several neurodegenerative/neuropsychiatric disorders.

1. GAPDH and Alzheimer disease (AD)

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by loss of neurons and formation of pathological extracellular deposits induced by amyloid-β peptide (Aβ)[16]. Numerous studies have established Aβ amyloidogenesis as a hallmark of AD pathogenesis[17], particularly with respect to mitochondrial dysfunction. Some scientist have proven that glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forms amyloid-like aggregates upon exposure to oxidative stress[18], and these aggregates contribute to neuronal cell death[19].Aggregated GAPDH in the brain is also amyloid genic, and GAPDH amyloidal aggregates localize with Lowy bodies in Parkinson's disease and with senile plaques and neurofibrillary tangles in Alzheimer's disease[20].

It has been posited that abnormal protein aggregation leads to mitochondrial dysfunction[21], proteasome inhibition[22], endoplasmic reticulum (ER) stress[23], and autophagy[24], which ultimately cause cell death. Notably, 5–20% of the total GAPDH under physiological conditions is generally bound to the mitochondria. Further, treatment of isolated mitochondria with GAPDH directly caused their dysfunction through the activation of voltage-dependent anion channels[25,26], which are known components of the mitochondrial permeability transition pore(PTP)[27] . PTP opening leads to mitochondrial depolarization and the release of cell death mediators from the intermembrane space, such as cytochromes (cytc) and apoptosis-inducing factor (AIF)[28]. From these observations, we focused on mitochondria to elucidate the neuronal cell death pathway that is mediated by GAPDH aggregation.

2. GAPDH and Huntington's disease

Huntington's disease (HD), is an autosomal, dominantly inherited, progressive neurodegenerative disease caused by CAG-trinucleotide repeat expansion (coding for a polyglutamine, polyQ, stretch) in the first exon of the gene IT15,[29] which encodes a large (~350 kDa) protein called huntingtin[30] and mutation in this gene leads to the production of an expanded polyglutamine stretch in the encoded protein[31], affecting ~5–10 per 100,000 people in the Western world[32]. Mutant protein, huntingtin, likely affects a wide range of cellular pathways and functions by abnormally interacting with a variety of proteins and intracellular organelles, thus contributing to the pathology underlying HD[33]. Patients affected by HD usually carry more than 35 repeats in their mutant huntingtin as compared to 16–20 repeats in the normal population[34].

After 10–20 years of onset of this disease, HD patients die due to complications from the disease [35]. Although the mutation in the huntingtin gene was identified more than 20 years ago[36], only symptomatic management[37]is currently available to treat the disease. Thus, it is important to identify the molecular mechanisms leading to the pathology associated with mutant huntingtin in HD and develop therapeutics targeting them. Mitochondrial dysfunction has been implicated in the pathology of HD because neurons, with high-energy demands, are dependent on mitochondria as a source of energy production to maintain their functions[38].

The dynamin family of GTPases (guanosinetriphosphatases) regulate the constantly repeated process of fission and fusion due to which mitochondria actively move from the neuronal soma to the axon and dendrites[39]. The mitochondria fission and fusion collectively refered to

as dynamics, are significantly disturbed and imbalanced in HD[40] leading to accumulation of fragmented and damaged mitochondria which ultimately leads to oxidative stress in cells. The cycle of fusion and fission is very closely coordinated with mitochondrial autophagy or mitophagy, a process of selective elimination of damaged mitochondria by autophagic machinery. Abnormalities at different phases of mitophagyin HD and in other neurodegenerative diseases have been proposed to contribute to disease progression through unknown mechanisms[41].

A new form of micro-mitophagy in which GAPDH associates with damaged mitochondria under oxidative stress induced by ischemia–reperfusion injury in hearts and promotes direct uptake of damaged mitochondria into the lysosomal structure that is composed of hybrid organelles of lysosomes and late endosomes, has been identified by one of the group. This process of micro-mitophagy occurs independently of the catalytic/metabolic activity of GAPDH [42]. In their study they sought to explore whether and how this GAPDH-driven mitophagy is regulated in HD. GAPDH is identified as one such protein which interacts with/affected by mutant huntingtin. It is reported that expression of mutant huntingtin with expanded polyglutamine repeats negatively regulates GAPDH-driven mitophagy thus contributing to HD-associated pathology. HD associated pathologyleading to accumulation of damaged mitochondria and inhibition of their removal by mitophagysuggests that it may also occur in other neurodegenerative diseases. Why macro-autophagy does not compensate for the impaired micro-autophagy it is still unclear. Whether GAPDH-mediated mitophagy is deployed together with a macro-autophagic pathway to remove damaged mitochondria and whether both pathways are impaired or inhibited with expression of mutant huntingtin with expanded polyglutamine repeats in HD are also unclear. GAPDH mediated mitophagy may also be incorporated into CMA, as Oxidized GAPDH is also a substrate of chaperone-mediated autophagy (CMA)[43,44,45] but this process still remains to be elucidated. Thus a better understanding of its mechanism will help with the elimination of damaged mitochondria that will allow us to prevent or slow down the disease progression. Under oxidative stress caused by expression of mutant huntingtin, GAPDH translocates to damaged mitochondria, which is an initial step toward cytoprotectivemitophagy. GAPDH at the outer mitochondrial membrane selectively associates with mutant huntingtin with expanded polyglutamine repeats, thereby blocking GAPDH mediated clearance of the damaged mitochondria by lysosomes[46]. By over expressing catalytically inactive GAPDH (iGAPDH), this mutant

huntingtin-induced impairment of mitophagy can be corrected; iGAPDH enhanced the blunted mitophagy and resulted in improved mitochondrial function and cell survival in cells expressing expanded polyglutamine repeats.

Micro-mitophagy provides a potential therapeutic approach to treat HD and maybe other neurodegenerative diseases as these data reflect a critical role of GAPDH-driven mitophagy in HD. But because of the complexity of the disease, a comprehensive treatment must also include a selective inhibitor of mitochondrial fission protein (Drp1) and a pharmacological activator of the macro-autophagic pathway such as mTOR-inhibiting drug rapamycin may provide the greatest therapeutic potential[47,48,49]. Also, some of the work shows that some DNA aptamers which bound to the elongated polyglutamine stretch of huntingtin with high affinity inhibited the aggregation of the protein in vitro. These aptamers succeeded in decreasing membrane permeabilization with concomitant reduction in intracellular oxidative stress[50]. They were also able to inhibit sequestration of an essential cellular enzyme, viz. GAPDH. Hence aptamers seems to be logical tool to slow down the progress of the disease by inhibiting the primary aggregation step.

3. GAPDH and Parkinson's disease (PD)

Parkinson's disease (PD) is a progressive age related neurodegenerative disorder with a prevalence of ~1% in people over 60years of age[51]. Pathologically it is characterized by the l together with the presence of proteinaceous cytoplasmic inclusions[52]. The majority of PD cases are apparently sporadic, while approximately 5–10% of the patients present an autosomal dominant or recessive mode of inheritance[53]. The etiology of sporadic PD is complex and multifactorial, involving aging, genetic and environmental risk factors. Over the years, candidate gene association studies have been extensively employed to identify loci where common variants contribute to the risk of PD[54]. Most recently a number of new susceptibility loci associated with PD have been identified through genome-wide association studies (GWAS)[55]. It has been found that GAPDH and glycosaminoglycans (GAGs) are associated with α-SN amyloid aggregates in Parkinson disease[56,57,58]. GAPDH has been seen to co-localize with α-SN in amyloid aggregates in post-mortem tissue of patients with sporadic Parkinson disease and promotes the formation of Lewy body-like inclusions in cell culture[59]. Some recent data suggests that GADPH also possesses highly diverse non-glycolitic functions in the intra or extracellular space[60] and has also been related to neurodegenerative diseases. GAPDH has a protective effect on late-onset Alzheimer disease[61]

which has been suggested by genomic analysis. If not all but GAGs are present in mosttypes of amyloids inside and outside of the cells [62,63]. Protein aggregation kinetics is affected by GAGs, which is proved in vivo[64]. It has been reported recently that sulfated GAGs, like heparin and heparansulfates, are able to trigger GAPDH amyloid aggregation under pH and temperature physiological conditions [65]. The GAPDH species induced by heparin formed during the early stages of the aggregation process (HI-GAPDH$_{ESS}$) are able to accelerate α-SN aggregation with a higher efficiency[66]. The interaction among GAGs, GAPDH, and α-SN exerts a protective role on dopaminergic cell survival[67].

GAPDH is critically involved in many other cellular processes including neurodegeneration apart from cellular energy metabolism. GAPDH protein plays an important role in dopaminergic neuronal apoptosis. GAPDH co-aggregates with α-synuclein which is the primary component of Lewy inclusion, which is confirmed from studies done on both cell models and post mortem brain tissues from sporadic PD. Abnormal aggregation and nuclear translocation of GAPDH protein were consistently observed in several in vitro apoptotic models induced by various neurotoxins including MPP, 6-OHDA and rotenone[68,69]. Furthermore, once the GAPDH were knock-downed, reduced cytotoxicity of these neurotoxins on dopaminergic cells were seen importantly, a recent in-vitro study in SHY-SY5Y showed that substitution of cysteine for serine-284 of human GAPDH led to aggregate prone GAPDH, which resulted in greater oxidative stress linked cell death than expression of wild type-GAPDH [70]. These findings suggest consistently that alteration in GAPDH function derived from genetic variation might be implicated in the pathogenesis of PD.

GAPDH is identified as a novel autoantigen that is expressed in neuronal cells at the plasma membrane level and is recognized by serum autoantibodies from patients affected with neurodegenerative disorder.[71] Serum IgGimmuno-reactivity to GAPDH was found in a high percentage of patients with major depression as compared to unaffected control individuals or patients with bipolar disorder[72]. Autoantibodies against GAPDH were also found in both the CSF and serum from patients with neuropsychiatric manifestations, suggesting that these antibodies, generated in the periphery, penetrate the CNS from the peripheral blood across the altered blood–brain barrier and bind to cell surfaces, possibly interfering with neuronal functions. GAPDH also has an important role in neurite outgrowth[73] which suggested that anti-GAPDH antibodies, when acting to block the binding of the molecule to laminin and/or to other adhesion and synaptic molecules in the CNS, may alter neuronal plasticity[74,75]. In

vivo administration of anti-GAPDH antibodies in C57BL6/J mice resulted in behavioral changes associated with a detrimental cognitive and emotional profile.

Current status

It is also investigated that the neuroprotective actions of deprenyl and TCH346 reflect their preventing GAPDH Siah binding and the nuclear translocation of GAPDH. It appears that the initial action of the drugs is to bind to GAPDH, as it is directly demonstrated binding of TCH346 to GAPDH[76]. Such binding would inhibit S-nitrosylation of GAPDH and its binding to Siah. Recently it is observed that rasagiline, a monoamine oxidase inhibitor used in the therapy of PD, is also neuroprotective in multiple animal models and prevents the nuclear translocation of GAPDH[77]. Although the principal focus for the therapeutic actions of deprenyl has been PD, deprenyl and related drugs might be useful in other neuronal conditions as well as non-nervous system conditions, because the GAPDH Siah cascade appears fairly universal [78, 79, 80].Thus a wide range of stressors in diverse cell lines elicits nuclear translocation of GAPDH with antisense to GAPDH preventing nuclear translocation and cell death. The most investigated GAPDH systems involve apoptotic death. Whether GAPDH plays a role in necrosis or in non-apoptotic programmed cell death is unclear. In addition to the therapeutic relevance, evidences supported cytoprotective actions of drugs involve blockade of the GAPDH Siah system provides support for the concept that the GAPDHSiah signalling cascade is an important component of cell death [81].

GAPDH is a redox-sensitive protein whose activity is largely affected by oxidative modifications at its highly reactive cysteine residue in the enzyme's active site (Cys149). Prolonged exposure to oxidative stress may cause, inter alia, the formation of intermolecular disulfide bonds leading to accumulation of GAPDH aggregates and ultimately to cell death.

Using the model of oxidative stress based on SK-N-SH human neuroblastoma cells treated with hydrogen peroxide it is observed that down- or up-regulation of GAPDH content caused inhibition or enhancement of the protein aggregation and respectively reduced or increased the level of cell death..The ability of the compounds to bind GAPDH molecules was proved by the drug affinity responsive target stability assay, molecular docking and differential

scanning calorimetry. Results of experiments with SK-N-SH human neuroblastoma treated with hydrogen peroxide showed that two substances, RX409 and RX426, lowered the degree of GAPDH aggregation and reduced cell death by 30%. Oxidative injury was emulated in vivo by injecting of malonic acid into the rat brain, and it was showed that the treatment with RX409 or RX426 inhibited GAPDH-mediated aggregation in the brain, reduced areas of the injury as proved by magnetic resonance imaging, and augmented the behavioral status of the rats as established by the "beam walking" test. In conclusion, the data show that two GAPDH binders could be therapeutically relevant in the treatment of injuries stemming from hard oxidative stress[82].

Novel evidences indicate that low molecular compounds may be effective inhibitors potentially preventing the GAPDH translocation to the nucleus, and inhibiting or slowing down its aggregation and oligomerization. Naturally occurring compound, piceatannol, to interact with GAPDH and to reveal its effect on functional properties and selected parameters of the dehydrogenase structure. The obtained data revealed that piceatannol binds to GAPDH. The ITC analysis indicated that one molecule of the tetrameric enzyme may bind up to 8 molecules of polyphenol (7.3 ± 0.9). Potential binding sites of piceatannol to the GAPDH molecule were analyzed using the Ligand Fit algorithm. Conducted analysis detected 11 ligand binding positions. It is indicated that piceatannol decreases GAPDH activity. Detailed analysis presume that this effect is due to piceatannol ability to assemble a covalent binding with nucleophiliccysteine residue (Cys149) which is directly involved in the catalytic reaction. Thus it is demonstrated that by binding with GAPDH piceatannol blocks cysteine residue and counteracts its oxidative modifications that induce oligomerization and GAPDH aggregation. Recently these anomalies have been linked with the pathogenesis of Alzheimer's disease[83].

Future Directions

GAPDH has become a subject of interest as studied revealed a surfeit of diverse GAPDH functions. As a result of multiple isoforms, cellular locales and diverse functions, GAPDH is able to come in contact with a variety of small molecules, proteins, membranes, etc., that play important roles in normal and pathologic cell function. Specially, GAPDH has been implicated in several neurodegenerative diseases and disorders, largely through interactions with other proteins specific to that disease or disorder.Various cell culture models have

conclusively demonstrated that initiation of apoptosis by a variety of ways involves an increase in the nuclear expression of GAPDH. This increased nuclear expression is dependent on the synthesis of new GAPDH protein. Whereas some GAPDH translocation from the cytosol may occur, this appears to be on a small scale and results in the activation of GAPDH transcription. This new GAPDH protein appears to have novel characteristics and is nuclear targeted. This nuclear-targeted GAPDH then regulates transcription to initiate PCD cascades. There is now accumulating evidence that these observations may have relevance to human neurodegenerative conditions. As such, recent reports have shown increased nuclear GAPDH associated with susceptible neurons in post-mortem samples from patients with a variety of neurodegenerative conditions. Small-molecule compounds with demonstrated anti-apoptotic activity that selectively interact with GAPDH have been identified. These compounds have also been shown to prevent the increase in nuclear GAPDH associated with the cell culture models in which they show anti-apoptotic activity. It is proposed that this effect is the result of binding to GAPDH, preventing the subsequent increase in GAPDH synthesis and nuclear accumulation. One of these compounds, CGP 3466, is currently undergoing Phase II clinical trials as a disease-modifying agent for Parkinson's disease, while others are in development.Thus, GAPDH may be used as an important therapeutic target in many neurological disorders like Parkinson disease, Alzheimer disease and Huntington's disease etc.

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