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Review Article

Oxidatively Modified Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and Alzheimer's Disease: Many Pathways to Neurodegeneration

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Abstract. Recently, the oxidoreductase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), has become a subject of interest as more and more studies reveal a surfeit of diverse GAPDH functions, extending beyond traditional aerobic metabolism of glucose. As a result of multiple isoforms and cellular locales, 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. Specifically, GAPDH has been shown to interact with neurodegenerative disease-associated proteins, including the amyloid-β protein precursor (AβPP). Studies from our laboratory have shown significant inhibition of GAPDH dehydrogenase activity in Alzheimer's disease (AD) brain due to oxidative modification. Although oxidative stress and damage is a common phenomenon in the AD brain, it would seem that inhibition of glycolytic enzyme activity is merely one avenue in which AD pathology affects neuronal cell development and survival, as oxidative modification can also impart a toxic gain-of-function to many proteins, including GAPDH. In this review, we examine the many functions of GAPDH with respect to AD brain; in particular, the apparent role(s) of GAPDH in AD-related apoptotic cell death is emphasized.

Keywords: Alzheimer's disease, amyloid-β, amyloid-β protein precursor, apoptosis, glyceraldehyde-3-phosphate dehydrogenase, hypometabolism, oxidative stress

INTRODUCTION

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzymes (EC 1.2.1.12) are a family of abundantly expressed oxidoreductases known for their role in glucose metabolism. Research on the role of mammalian GAPDH in various biological signaling pathways over the last couple of decades has increased interest in this classical glycolytic enzyme. Traditionally, GAPDH was used as a model, or control, in protein and gene structural- and catalytic mechanism-related studies, as well as a standard in Northern and Western blots because of its high degree of gene and protein sequence conservation across species [1–3].

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However, recent research suggests that GAPDH possesses highly diverse, non-glycolytic functions, as its expression and activity are affected by multiple factors. In particular, GAPDH has been reported to bind DNA and RNA [1,4–6], regulate transcription [7], possess kinase/phosphotransferase activity [8–15], catalyze microtubule formation and polymerization [16–22], facilitate vesicular transport [23], and bind integral membrane ion pumps associated with Ca^{2+} release [24,25], as well as interact with a number of small molecules, including tumor necrosis factor (TNF)-α ribozymes [26], glutathione (GSH) [27,28], p53 [29–31], and nitric oxide (NO) [32–34]. Additionally, since GAPDH also interacts with disease-associated proteins, like huntingtin [35] and the amyloid-β protein precursor (AβPP) [36]. GAPDH non-glycolytic activity is of great interest to neurodegenerative disease research, specifically in brains of subjects with Alzheimer's disease (AD).

AD is the most common age-related neurodegenerative disorder affecting elderly populations over the age of 65. In familial cases, however, AD pathology can present as early in life, due to autosomal dominant mutations in the AβPP protein and *presenilin* genes 1 and 2 [37–40]. Characteristic symptoms of AD include progressive memory loss, declining cognition, impaired linguistic function, and dementia. Pathologically, the brain exhibits extensive synapse and neuronal cell loss, as well as the appearance of neurofibrillary tangles (NFT) and senile plaques, associated with widespread oxidative stress and damage [41–47]. Studies from our laboratory demonstrate that GAPDH is subject to many different types of oxidative modification in AD brain, which drastically affect its structure and function, including S-glutathionylation [28,34,48], S-nitrosylation [49–51], and direct or indirect reaction with reactive oxygen species (ROS) [52–55]. Moreover, a recent study by Petrak and colleagues [56] found that the frequency with which GAPDH was shown differentially expressed in all 2D-gel electrophoresis (2-DE)-based experiments in human and rodent tissues was $\sim$18%, securing it a spot on the top 15 list of most frequently reported differentially expressed proteins. Taking into consideration the multitude of functions GAPDH can carry out under normal conditions, in addition to a variety of subcellular locations, it is not surprising that this enzyme is so often affected by disease pathology. In this review we will discuss the different roles of GAPDH, and how those roles are affected by and/or contribute to neurodegenerative disease; our focus will be on understanding the function(s) of GAPDH in AD.

GAPDH STRUCTURE

GAPDH (EC 1.2.1.12) is a member of the dehydrogenase enzyme family, also known as oxidoreductases, and is essential to glucose metabolism. This glycolytic enzyme is ubiquitously expressed in both prokaryotes and eukaryotes, and comprises $\sim$10–20% of the total cellular protein content [1]. All mammalian GAPDH genes, including human, have a complex genetic organization; gene expression studies reveal that human GAPDH has only one functional gene located on chromosome 12 (Gene ID: 2597), but around 150 or more pseudogenes with similar sequence identity [57–61]. Further investigation reveals the presence of a single GAPDH mRNA species in different tissues [57,59–61].

GAPDH exists as a homologous tetramer ($\sim$150 kDa), monomer, and dimer, of which there is little information at present [8,62]. The tetrameric form is located mainly in the cytoplasm and is comprised of four chemically identical subunits, O, P, Q, and R (Fig. 1), each approximately 37 kDa, with three asymmetric interfaces between subunits P, Q, and R [63,64]. The monomeric form ($\sim$37 kDa) is localized to the nucleus, mainly during cell proliferation, and consists of 335 amino acids [65,66]. Each GAPDH monomer contains two binding domains: an N-terminal NAD^+-binding domain and a C-terminal catalytic, or glyceraldehyde-3-phosphate (G3P)-binding domain. The NAD^+-binding domain contains amino acid residues 1–151, forming the main-chain, while the G3P-binding domain consists of residues 315–335, forming the C-terminal helix. The NAD^+-binding domain, or coenzyme domain, also contains the well-known Rossmann fold structure necessary for binding dinucleotides. Interestingly, a number of active-site amino acids (human analogs, Asp-35, Cys-152, His-179, Thr-182, Thr-211, Arg-234, Tyr-314, Tyr-320) that are directly involved in binding of the G3P and NAD^+ nicotinamide moiety and responsible for catalytic activity are part of a highly conserved sequence, that, on the phylogenic scale, maintains a highly conserved 3D-structure (Fig. 2) [2]. Conversely, the structure surrounding the adenine and phosphate binding sites vary across species [2,3,67,68].

Under normal conditions, the NAD^+ molecule situates in a cleft formed by the coenzyme domain S-loops from adjacent subunits along the R-axis (Fig. 1); NAD^+ binds to the C-terminal edge of a parallel β-sheet, flanking it between two α-helices. As the nicotinamide end of NAD^+ faces the interior of the GAPDH tetramer, a cleft opens, revealing a spacious active-site pocket (Fig. 1) [2]. The catalytic domain, on the oth-

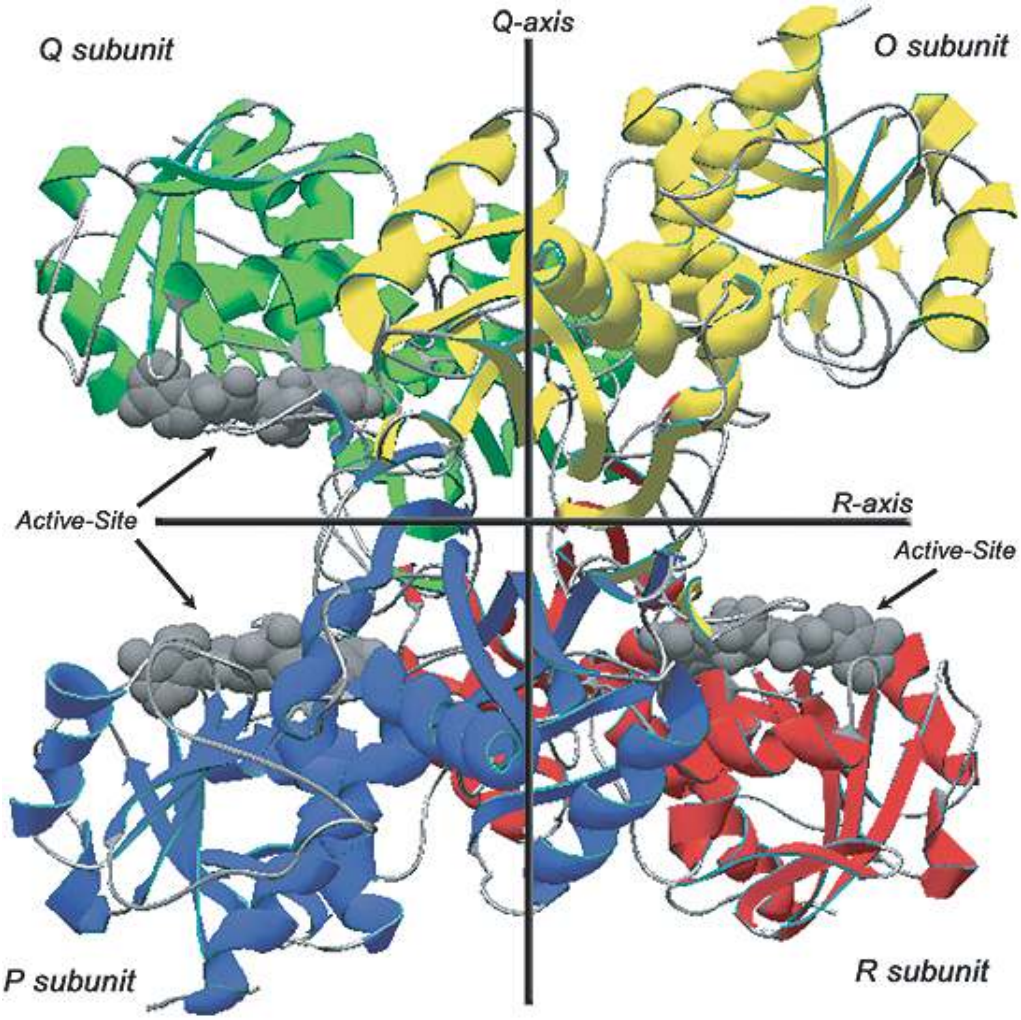

Fig. 1. *GAPDH Structure*. Ribbon view of the human placental GAPDH, subunits O, P, Q, and R. GAPDH active-sites clefts are indicated by arrows on each of the asymmetric subunits, P, Q, and R. Color codes: Green, subunit Q; Yellow, subunit O; Blue, subunit P; Red, subunit R; Gray, backbone carbon loops connecting secondary structure successions; Gray molecular structures, NAD(+). Data were obtained from the RCSB protein data bank as entry code 1u8f.pdb, DOI: 10.1107/S0907444905042289 [2]. This figure was drawn using the DeepView Swiss-PDB viewer program, version 4.0.

er hand, consists of twisted, eight-stranded parallel β-sheets connected by α-helices on one side, while the other side of this β-sheet forms extensive contacts with the β-sheet of an adjacent subunit (Fig. 1) [2,69,70]. In addition, the amino acid Val-240 (human analog) of each subunit falls in a disallowed region of Ramachandran plots, which is a characteristic of all GAPDH enzymes [70–74].

GAPDH ACTIVE-SITE FUNCTION

GAPDH is most well-known for its role in glycolysis, catalyzing the reversible phosphorylation of G3P to 1,3-bisphosphoglycerate (BPG) using NAD^+ as a cofactor (Fig. 3). The first step of this mechanism involves nucleophilic attack by the sulfhydryl group of Cys-152 on the carbonyl of G3P, resulting in the formation of a hemiacetal (Fig. 4). The hemiacetal intermediate is then oxidized to a high energy thioester, by hydride transfer to NAD^+, possibly facilitated by His-179 in the catalytic domain [75,76]. This high energy thioester is then attacked by nucleophilic, inorganic phosphate (P_i) to form BPG (Fig. 4) [68,69, 77]. Mutational studies in Bacillus stearothermophilus show replacement of Cys-149 (human analog Cys-152) with a Ser thiol significantly reduces GAPDH activity,

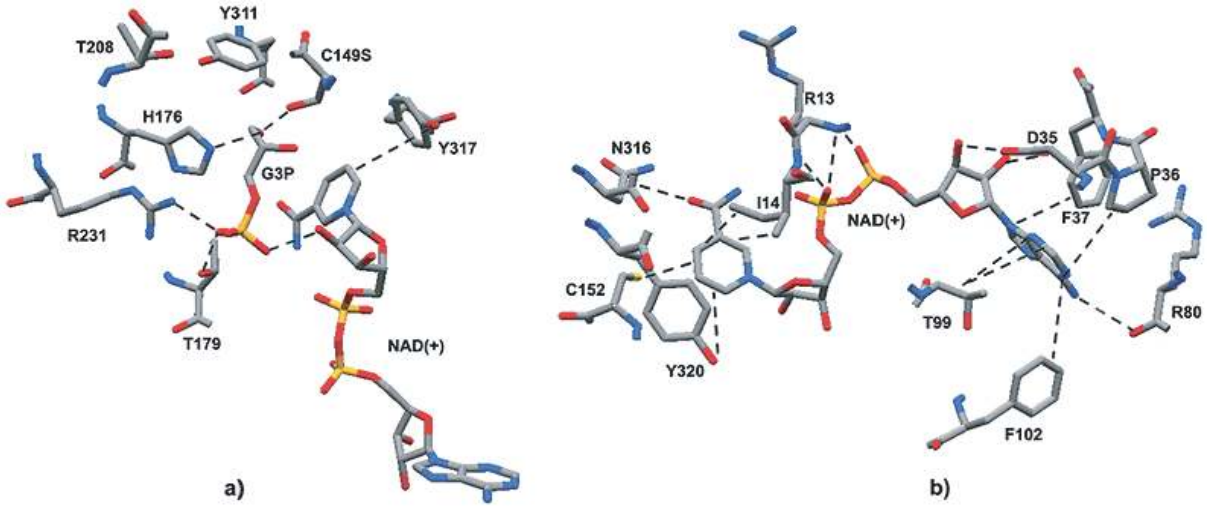

Fig. 2. *GAPDH active-site*. a) View of *Bacillus stearothermophilus* GAPDH active-site catalytic residues with a Cys149Ser substitution and occupied by D-G3P and NAD(+). Residues shown are those directly involved in G3P binding (human amino acid analogs). The structure was drawn in CPK format: Gray, carbons atoms; Blue, nitrogen atoms; Red, oxygen atoms; Orange, phosphorous atoms. Dotted lines represent potential electrostatic or non-polar interactions between G3P, NAD(+), and amino acids; residues T208 and Y311 are normally directly involved with binding of the C149 sulfate anion, however, the C149S substitution replaces the SH-group with a hydroxyl, as shown. Data were obtained from the RCSB protein data bank as entry code 1NQO.pdb, DOI: 10.1074/jbc.M211040200 [77]. This figure was drawn using the DeepView Swiss-PDB viewer program, version 4.0. b) View of human placental GAPDH active-site catalytic residues with no substitution and occupied only by NAD(+). Residues shown are those directly involved in NAD^+ binding. The structure was drawn in CPK format: Gray, carbons atoms; Blue, nitrogen atoms; Red, oxygen atoms; Orange, phosphorous atoms; Yellow, sulfur atoms. Dotted lines represent potential electrostatic or non-polar interactions between NAD(+) and specified amino acids. Data were obtained from the RCSB protein data bank as entry code 1u8f.pdb, DOI: 10.1107/S0907444905042289 [2]. This figure was drawn using the DeepView Swiss-PDB viewer program, version 4.0.

while substitution with Ala completely inactivates the enzyme [77,78].

GAPDH has a very large active-site cavity in order to accommodate G3P, with its bulky phosphate ions, and the cofactor NAD^+, located in a large cleft between the NAD^+ – and G3P-binding domains (Fig. 1). Structural analysis of bacterial and eukaryotic GAPDH reveals that there are two anion (i.e., phosphate) binding sites located in the catalytic domain that bind a P_i and the C_3-phosphate of the substrate, G3P (Fig. 2b) [79]. Literature studies show that Arg-234 (bacterial analog Asp-231) of catalytic domain acts as a conformational switch that regulates G3P binding and release (Fig. 2a), as chemical modification of Arg-234 results in a 95% reduction in GAPDH activity [77,79–81]. Moreover, in the catalytic domain, His-179 forms a hydrogen bond with human Cys-152 (Fig. 2a), which resides at the N-terminus of the first helix of the catalytic domain, where it acts as a base catalyst to facilitate hydride transfer [2]. In the NAD^+-binding domain, nicotinamide carbonyls form hydrogen bonds with Asn-316 (human analog), while nicotinamide amines form intramolecular hydrogen bonds with its pyrophosphate groups in the N-terminal, glycine-rich, first helix loop of the Rossmann fold (Fig. 2b) [2]. Finally, a highly conserved Asp-35 residue forms two hydrogen bonds with the adenosine ribose moiety of NAD^+, while a nonpolar interaction between NAD^+ and Ile-14 and Tyr-320 (human analogs) holds the nicotinamide ring in place (Fig. 2b) [2].

GAPDH FUNCTIONAL DIVERSITY

Apart from a classical role in glycolysis, GAPDH also exhibits numerous non-glycolytic functions, in which the NAD^+- and G3P-binding domains play a very important role. Analysis of such functions after proteolysis and competitive binding using NAD^+ and polynucleotides indicates that the majority of GAPDH functional diversity is a direct result of the many different interactions that can occur within the NAD^+-binding domain [1,26,82–85]. For example, the NAD^+-binding domain Rossmann fold, essential to the dehydrogenase activity of GAPDH [2,69,70], is also important in the catalysis of aminoacyl tRNA synthesis by GAPDH [86], and may comprise the GAPDH nucleotide binding site for tubulin [87]. Moreover, GAPDH can act as a nuclear tRNA transport protein,

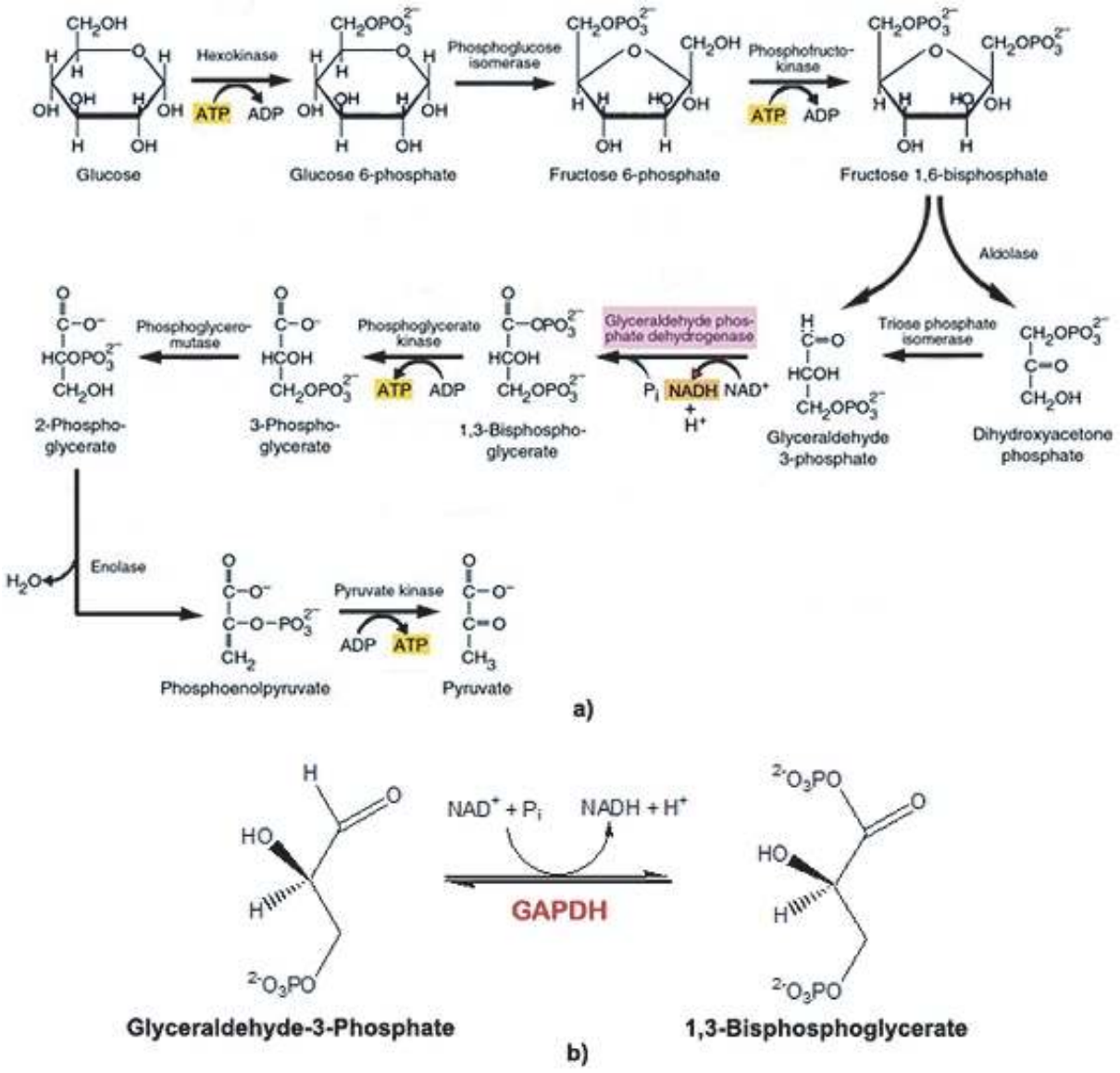

Fig. 3. a) *Glycolysis*. Schematic representation of glucose metabolism, with reactions 5-10 completed twice (not shown) (adapted from [224]). b)*Overall GAPDH reaction*. The sixth reaction in glycolysis involves conversion of glyceraldehyde-3-phosphate (G3P) to 1,3-bisphosphoglycerate (BPG) by GAPDH, using NAD^+ as a cofactor.

whose activity is inhibited by NAD^+ in a competitive manner [1,88].

In addition, experimental evidence suggests that the catalytic G3P-binding domain is involved in regulation of GAPDH membrane binding and subcellular localization in mammalian cells. A study with human erythrocyte membranes shows that modification of G3P-binding site residues, Lys-191 and Lys-212, may determine GAPDH-membrane interactions [1,89]. Moreover, amino acid sequence analysis of GAPDH reveals the presence of sequence motifs that are often used by mammalian cells to regulate the intracellular localization of a protein. One motif has the sequence KKVVK (residues 259–263), which is partially homologous to the nuclear localization signal (NLS), and the other is ALQNIJP (residues 202–208), which is partially homologous to a nuclear export domain [1,90]. In contrast, other observations suggest GAPDH may not require such a NLS, since proteins as small as monomeric GAPDH do not require active transport [91–93]. Re-

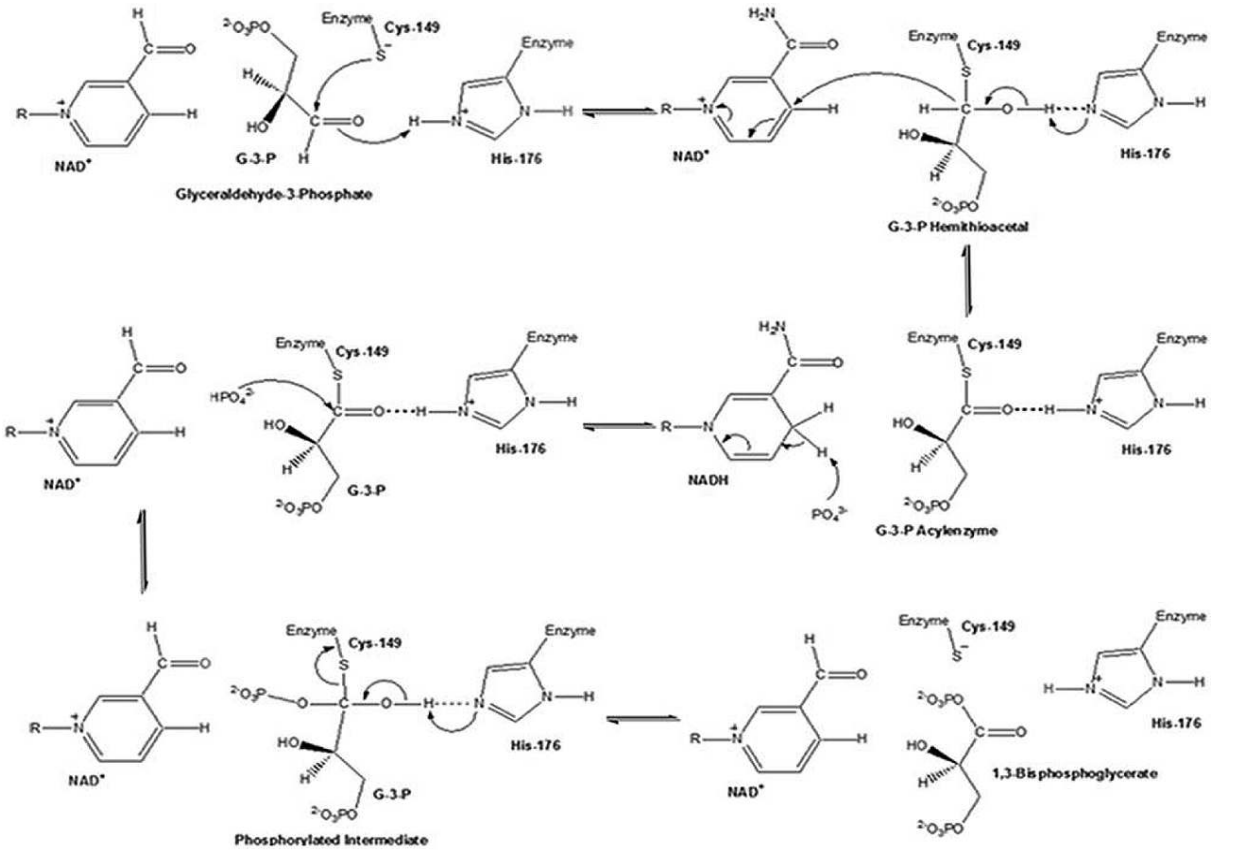

Fig. 4. *GAPDH Reaction*. The reaction catalyzed by GAPDH involves two processes: Oxidation of the glyceraldehyde-3-phosphate (G3P) aldehyde to a carboxylic acid by NAD^+, and joining of the G3P carboxylic acid and the NAD^+ orthophosphate to form 1,3-bisphosphoglycerate (BPG). The first step of this mechanism involves nucleophilic attack by the Cys-149 (human analog Cys-152) SH-group on the carbonyl of G3P, resulting in formation of a hemithioacetal. Acting as an acid catalyst, His-176 (human analog His-179) facilitates hemiacetal development by forming an ion pair with the Cys-149 SH-group, enhancing Cys-149 reactivity, and favoring the nucleophilic attack of GAPDH by the thiolate [76]. A hydride transfer to NAD^+ oxidizes the hemiacetal intermediate to a high energy thioester, facilitated by His-176, now acting as a base catalyst during the oxidation step [75,76]. His-176 stabilizes the hemithioacetal intermediate by hydrogen bonding the hemiacetal hydroxyl group to the N^{ε} of its imidazole ring, while the acylenzyme intermediate, is stabilized by hydrogen bonding with the protonated imidazole N^{ε} of His-176 [76]. Finally, the thioester undergoes nucleophilic attack by an inorganic phosphate (P_i), facilitated again by His-176 acting as an acid catalyst, to form BPG [75–77], which is then released from the ternary complex, along with NADH (adapted from [76]).

gardless of the mode of entry, monomeric GAPDH can be found in the nucleus of non-apoptotic cells [1,65]. Based on this information, it can be suggested that the NAD^+-binding domain is associated with GAPDH functional diversity, while the G3P-binding domain is responsible for GAPDH intracellular localization [1].

Nuclear/perinuclear GAPDH function

In general, non-glycolytic functions of GAPDH can be divided into nuclear, perinuclear, cytosolic, and membrane-related functions. As part of its nuclear and/or perinuclear activities, monomeric GAPDH interacts with DNA and RNA. Several studies report that GAPDH binds DNA [4] and exhibits DNA repair activity similar to a uracil DNA-glycosylase (UDG), a DNA repair enzyme that removes free uracils [1,5,6]. *In vivo* studies have also shown that suppression of nuclear UDG activity increases GAPDH mRNA levels and dehydrogenase activity [94]. GAPDH can also interact with the 5'-UTR and 3'-UTR regions of various mRNA sequences through its NAD^+-binding domain [4]. A dose-response study with human parainfluenza virus shows that an increase in NAD^+ decreases the ability of GAPDH to bind RNA by competitive inhibition [83]. Other nuclear/perinuclear functions of GAPDH include the ability to bind the Octomer binding protein-1 (Oct-1) involved in histone transcription [7], increase the activity of TNF-α ribozymes by interacting with their pyrimidine regions [26], and bind the dinu-

cleoside phosphate, P^1, P^4-di(adenosine-5') tetraphosphate (Ap4A) protein, which plays a significant role in DNA replication and repair [5,95], among others (Table 1). Moreover, since the ratio of Ap3 to Ap4 changes in apoptosis, GAPDH is thought to modulate Ap4A protein functions at various stages of the cell-cycle [4, 5,96].

Interestingly, ROS has been shown to play an important role in modulating non-glycolytic nuclear/perinuclear functions of GAPDH. In HEK-293T cells expressed with human GAPDH, Hwang and coworkers [97] have shown that active-site cysteine residues (human analog Cys-152) of GAPDH are oxidized upon H_2O_2 treatment, forming cysteic acid and/or intramolecular disulfide bonds with Cys-156 depending on the extent of oxidative stress. Thus oxidized GAPDH interacts with heterodimeric RNA and DNA-binding proteins, 54 kDa RNA- binding protein (p54nrb) and polypyrimidine tract-binding protein-associated splicing factor (PSF), enhancing topoisomerase-I activity. Involvement of GAPDH in modulation of topoisomerase-I activity during DNA replication and transcription, together with a multitude of other diverse nuclear/perinuclear GAPDH functions, strongly suggests GAPDH may also function as a transcription factor [97].

Cytosolic GAPDH functions

As described above, the most common GAPDH isoform found in the cytoplasm is tetrameric. In addition to its glycolytic activity, previous studies have confirmed numerous kinase and phosphotransferase activities elicited by cytosolic GAPDH, other than phosphorylation of G3P. First, dimeric GAPDH has been reported to associate with the tumor suppresser nucleoside diphosphate protein kinase, nm23 [8]. In order to elicit Ser/Thr phosphotransferase activity in human cells, nm23 must first complex with dimeric GAPDH, as nm23 alone shows no kinase activity [8]. Interestingly, the glycolytic activity of GAPDH was not inhibited by this interaction, suggesting that the NAD^+- and/or G3P-binding domains are vital to GAPDH phosphotransferase/kinase function. In addition, mammalian GAPDH has been shown to phosphorylate the Hepatitis B virus (HBV) in a Ca^{2+}-independent manner, opening new avenues investigating the role of GAPDH in viral pathogenesis [9].

Another facet of its phosphotransferase/kinase activity involves phosphorylation of GAPDH by various cellular kinases including, protein kinase C [10,11], epidermal growth factor kinase [12], and Ca^{2+}/calmodulin-dependent protein kinase II [13], among others (Table 1). The exact GAPDH amino acid modified by these kinases is unknown; however, preliminary studies suggest a Tyr as the site of phosphorylation [13, 98]. Furthermore, studies have shown that mammalian, tetrameric GAPDH was auto-phosphorylated in the presence of ATP and Mg^{2+} in a concentration-dependent manner and dephosphorylated in the presence of NAD^+, NADH, and/or G3P [9,14,15]. Although, the role of GAPDH phosphorylation in normal cell function is unclear at present, it would appear that auto-phosphorylation is not necessarily a prerequisite to its other known phosphotransferase or kinase activities.

Beyond phosphorylation, cytosolic GAPDH also catalyzes microtubule formation and polymerization by binding the cytoskeletal protein tubulin [16–22], binds to the mitochondrial voltage-dependent anion channel protein (VDAC-1) [99], associates with small molecules like GSH [27,28], p53 [29–31], NO [32–34], and interacts with proteins associated with neurodegenerative disease, such as the huntingtin protein in Huntington's disease [35], the androgen receptor protein in spinocerebellar ataxia type-1 and spinobulbar muscular atrophy [100], and AβPP and amyloid β-peptide (Aβ) in AD (Table 1) [36,101,102], to be discussed in this review.

Membrane-associated GAPDH activity

As part of its membrane-associated activities, earlier studies revealed that GAPDH binds to the human erythrocyte membrane [103] and is part of the phospholipid bilayer [1,104]. This enzyme is also involved in vesicular transport from the Golgi apparatus to the endoplasmic reticulum (ER) [23], and has Ca^{2+}-dependant fusogenic activity in human neutropil [105] and rat brain cytosol (Table 1) [106]. Previous studies demonstrated that fusogenic activity was dependent on Ser-234 [107], and inhibited by the substrate G3P. Interestingly, GAPDH fusogenic activity was inhibited by G3P, but not affected by the dehydrogenase inhibitor, koningic acid, as those GAPDH isoforms displayed fusogenic activity, but were void of dehydrogenase activity [106]. Furthermore, GAPDH has been shown to bind integral membrane proteins, such as the inositol-1,4,5-triphosphate receptor (IP3R) and sarcoplasmic reticulum Ca^{2+} (SERCA) pump (Table 1) [24,25], contributing to the regulation of intracellular Ca^{2+} levels.

Table 1
GAPDH functional diversity

Cytosolic Functions:

– Glycolytic/Gluconeogenesis enzyme
– Metabolic switch between Glycolysis and Pentose Phosphate Shunt
– Kinase/Phosphotransferase
– Undergoes auto-ADP-ribosylation
– Microtubule bundling
○ Tubulin binding
○ Binding and stabilization of fully formed microtubules
– Endocystosis
– Fusogenic membrane-bound protein
– Other intracellular protein binding:
○ NO sensor
○ Glutathione (GSH)
○ TNF-α
○ p53
○ VDAC
○ Ap4A
○ nm23
○ Siah-1(in apoptosis)
○ IP3 receptor
○ SERCA pump
○ HSP70 (in ischemia and stroke)
○ Alzhiemer's-related proteins:
■ AβPP
■ Aβ_{1-40} and Aβ_{1-42}
■ Tau
○ Huntington's-related proteins:
■ Huntingtin
○ Parkinson's-related proteins:
■ $\alpha-$synuclein
■ Lewy bodies
■ Parkin
○ Other triplet repeat disorders:
■ Atrophin in Dentratorubapallidoluysian atrophy (DRLPA)
■ Ataxin-1 in Spinocerebellar ataxia, type-1
■ Androgen receptor in Spinobulbar muscular atrophy
■ Granular deposits in Spinocerebellar ataxia, type-3 (Machado-Joseph disease)

Nuclear Functions:

– DNA replication
– Uracil DNA-glycosylase
– Nuclear RNA transport
– Translational regulation
○ Catalysis of amino acyl tRNA synthesis
– Transcription factor
○ Oct-1 binding in histone transcription

Other Disease Involvement:

– Metal toxicity (?)
○ Zinc binding in zinc neurotoxicity
– Phosphorylates Hepatitis B virus in HBV viral pathogenesis
– Swine fever (?)
– Cancer:
○ Prostate cancer
○ Pancreatic cancer
○ Acute lymphoblastic leukemia

(?), signifies unknown/debated GAPDH functions and/or disease involvement (adapted from [1,30]).

GAPDH AND ALZHEIMER'S DISEASE

GAPDH can undergo many different oxidative modifications, which influence its structure and activity. Active-site modifications include, GAPDH-transition metal complex formation [108], mono-ADP-ribosylation [50,109], *S*-glutathionylation [28,34,48], *S*-nitrosylation by NO or other reactive nitrogen species (RNS) [49–51], and direct or indirect reaction with ROS, as measured by levels of protein carbonylation and modification by the lipid peroxidation product, 4-hydroxy-2-nonenal (HNE) [52–55]. In AD brain, such oxidative modifications are common, as numerous studies have shown oxidative stress and damage to be a major hallmark of AD pathology, precipitating the loss of neurons, synapses, and ultimately, normal brain function [41–47]. Studies conducted by our laboratory have revealed at least 42 proteins negatively affected by the shifting redox environment in AD brain, including GAPDH (Fig. 5) [48,51,52,54,55]. Furthermore, a study by Petrak and colleagues [56] found that the frequency with which GAPDH was shown differentially expressed in all 2D-gel electrophoresis (2-DE)-based experiments in human and rodent tissues was $\sim$18%, securing it a spot on the top 15 list of most frequently reported differentially expressed proteins. Considering the surplus of different GAPDH functions and locations within the cell, as well as the frequency with which it is differentially expressed, oxidative dysfunction of GAPDH may significantly contribute to loss of neuronal function and neurodegeneration in AD brain.

GAPDH and apoptosis

Though somewhat controversial, increasing evidence suggests apoptosis occurs in AD brain [110–116]. Apoptosis involves a series of intracellular signaling events that ultimately lead to programmed cell death in response to relatively mild stimuli [117]. Tetrameric GAPDH stability is achieved by ionic interactions between positively charged NAD^+ and negatively charged sulfate ions of active-site Cys residues. Modification of these cysteines decreases the tetramer's stability, giving rise to monomers, dimers, and other denatured GAPDH products [62,118], that individually elicit various levels of glycolytic function [119]. Interestingly, all GAPDH isozymes have been shown to be altered during apoptosis [120]. Numerous evidence supports the role of GAPDH in apoptosis, facilitating cell death induced by apoptotic stimuli and/or oxidative stress by nuclear translocation [30]. The first evidence of this relationship was observed in rat cerebellar granule and cortical neuronal cell cultures undergoing spontaneous apoptosis. In this study, it was found that monomeric GAPDH was overexpressed prior to apoptosis, while transfection with antisense GAPDH caused a significant reduction in apoptosis concomitant to reduction of GAPDH mRNA [121,122]. However, even though these studies establish a role for GAPDH in apoptotic processes, how GAPDH participates is still a subject of much research.

While investigating mechanisms of GAPDH apoptotic function, studies have found that GAPDH acts as a pro-apoptotic protein after nuclear translocation. Analysis of cerebellar granule and cortical neuronal cell culture after cytosine arabinoside (AraC)-induced apoptosis showed increased levels of GAPDH in mitochondrial and crude nuclear fractions [121,122]. Because treatment with antisense GAPDH completely blocked AraC-induced apoptosis, it is probable that GAPDH translocation functions as a potent initiator of apoptotic processes [121–123]. Interestingly, nuclear GAPDH accumulation was allied with a reduction in GAPDH UDG activity, as glycolytic activity initially increased in response to AraC treatment and decreased with UDG activity [6,120]. With respect to AD brain, these studies present two ways in which GAPDH could be involved in disease pathology: First, because GAPDH is a known transcription factor [7], nuclear translocation of cytosolic GAPDH could induce transcription of genes that mediate cell death. Second, suppression of GAPDH UDG activity after translocation would prevent DNA repair [5,6,94], thereby, increasing levels of damaged DNA, a common finding in apoptosis-related cell death. Nuclear translocation and accumulation of GAPDH also occurs with concomitant degradation of lamin B1, a nuclear membrane protein and caspase-3 substrate. Analysis of purified nuclei showed an increase in the levels of six GAPDH isoforms after AraC treatment, the greatest increase of which was found in more acidic isoforms [120]. Thus, the significance of acidic isozymes in neuronal apoptosis, in addition to the differential translocation behavior of different GAPDH isoforms, strongly suggests a key role for cytosolic GAPDH translocation in AD-related apoptotic processes.

A second way GAPDH is involved in apoptosis is through post-translational modification and small molecule/protein interactions, including Ap4A binding [5,124], VDAC-1 binding [99], phosphorylation [8–15,98], *S*-glutathionylation, p53 binding, *S*-nitrosylation, and AβPP binding. Ap4A is a part

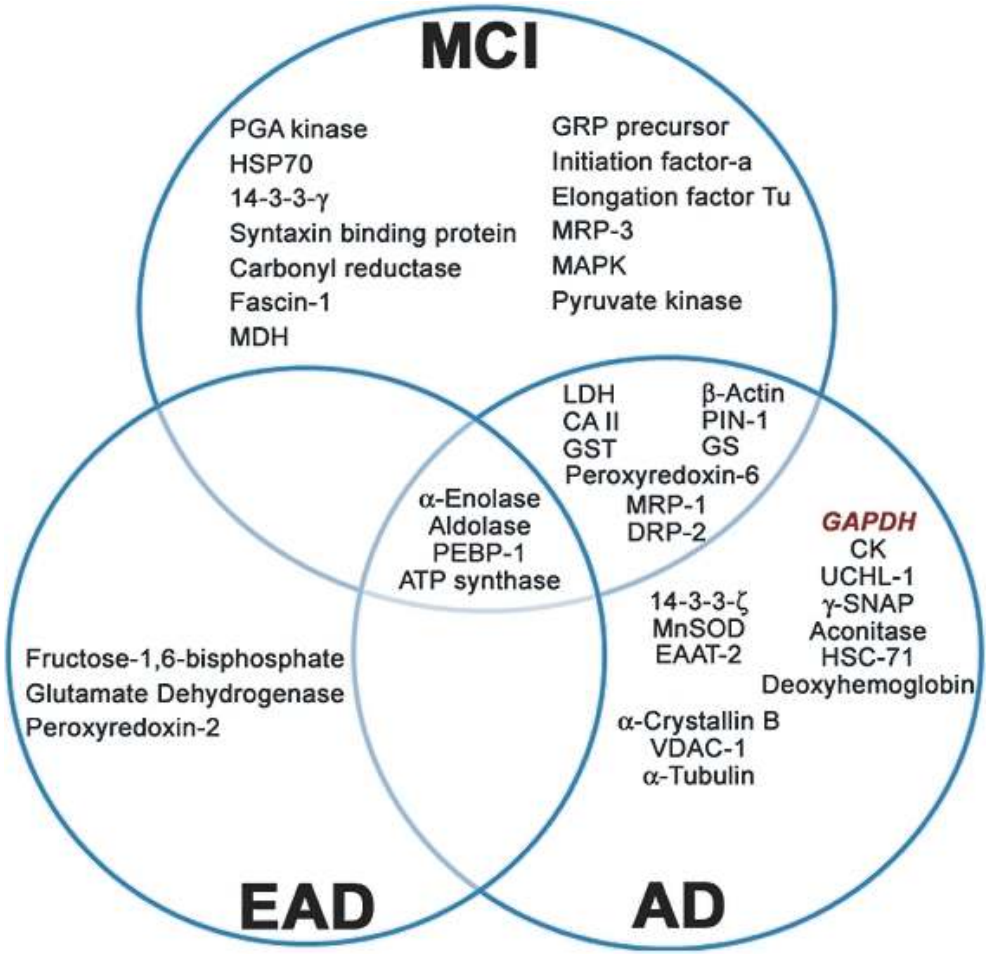

Fig. 5. *Proteins oxidatively modified in MCI, EAD, and AD brain identified by our laboratory* [48,51,55,225–231]. The inter-relationship of those proteins identified to be significantly modified by protein carbonylation, HNE- and 3-NT modification, as well as S-glutathionylation using redox proteomics on mild cognitive impairment (MCI), early AD (EAD), and AD human brain are shown. Abbreviations: PCG kinase, phosphoglycerate kinase; HSP-70, Heat-shock protein-70; MDH, Malate dehydrogenase; GRP precursor, Glucose-regulated protein precursor; MRP-1/MRP-3, Multidrug-resistant protein-1 or -3; MAPK, Mitogen-associated protein kinase; PEBP-1, phosphatidylethanolamine-binding protein-1; LDH, Lactate dehydrogenase; CAII, Carbonic anhydrase II; GST, Glutathione S-transferase; DRP-2, Dihydropyrimidinase-related protein-2; PIN-1, Peptidyl-prolyl cis/trans isomerase; GS, Glutamine synthetase; MnSOD, Manganese superoxide dismutase; EAAT-2, Excitatory amino acid transporter-2; VDAC-1, Voltage dependent anion channel-1; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; CK, Creatine kinase; UCHL-1, Ubiquitin carboxy-terminal hydrolase L-1; γ -SNAP, Soluble N-ethylmaleimide-sensitive factor attachment protein-γ; HSC-71, Heat-shock cognate-71.

of the diadenosine oligophosphate (ApnA) signal-transduction family of molecules, which play a role in DNA replication and repair through binding cell membranes and the DNA polymerase-α complex [5, 95,124]. Ap4A may act as a neurotransmitter (see review [125]). Previous reports have shown that Ap4A is neuroprotective against 6-hydroxydopamine-induced neurotoxicity in a rodent model of Parkinson's disease [126]. Moreover, pre- and post-treatment with Ap4A was shown to protect neurons against hypoxic and ischemic brain injury [126,127]. In other investigations, however, these diadenine nucleotides have also been linked to apoptosis. One study of human cell cultures reported that apoptosis was associated with decreased levels of Ap3A and increased levels of Ap4A [95,128]. Furthermore, in HeLa cell studies, Mg^{2+}-dependent GAPDH-Ap4A binding was observed using photo affinity probes, and later confirmed by gel filtration and SDS-PAGE analysis [4,5]. Unfortunately, the role played by the association of GAPDH and Ap4A in apoptosis, let alone in AD, is unclear at present; however, its significance with respect to the

role of Ap3A/Ap4A ratios in programmed cell death is a subject of ongoing investigation.

Interestingly, it has been reported that GAPDH can also be found in and bound to mitochondria, generally by association with the mitochondrial membrane protein, VDAC-1, a component of the mitochondrial permeability transition pore complex (MPTP) [99, 119]. Upon binding GAPDH, the MPTP opens, triggering inner mitochondrial membrane permeabilization, the loss of transmembrane potential, increased cytosolic Ca^{2+} uptake, matrix swelling, and the release of cytochrome C and apoptosis inducing factor (AIF) into the cytosol. In effect, this cascade is the beginning of an intrinsic apoptotic process initiated by GAPDH. It should be noted, however, that although GAPDH is present in mitochondria and enzymatically active under normal conditions [119,129], overexpression of GAPDH and its import into mitochondria via VDAC-1 is required to trigger an apoptotic cascade through the MPTP [99]. In a study by Tarze et al. [99], it was suggested that the GAPDH-VDAC-1 interaction occurred via inter- and/or intra-molecular disulfide bonding, as their association was inhibited by administration of the thiolating agent, dithiothreitol (DTT). Moreover, these researchers speculate that monomeric GAPDH isoforms were the most likely candidates involved in pro-apoptotic MPTP opening, as denaturing conditions did not interfere with GAPDH-VDAC-1 binding [99]. Likewise, it would also appear that enzymatic activity was not a prerequisite for VDAC-1 binding [99]. The opening of the MPTP in neurodegenerative disease is well know (reviewed in [130]), and favored by the overwhelmingly oxidative environment in AD brain. Although whether or not oxidized GAPDH binds VDAC-1 was not described, the aforementioned study [99] strongly suggests the likelihood of their interaction, considering monomeric and/or other denatured isoforms of GAPDH readily associate with VDAC-1. Thus, it can be inferred that the pro-apoptotic GAPDH-VDAC-1 interaction also could lead to AD pathology.

Other post-translational modifications that may link GAPDH to apoptotic processes involve its phosphotransferase/kinase activity. As a phosphotransferase, or kinase, GAPDH is able to substitute the hydrogen atom of a hydroxyl moiety on Ser, Thr, and Tyr residues with a highly negative phosphate group, causing significant conformational changes in the phosphorylated protein's structure [1]; likewise, GAPDH structure is also dramatically altered when phosphorylated by other intracellular protein kinases. Phosphorylation is one of the most common post-translational modifications the cell employs to complete a myriad of signaling cascades under normal conditions. However, a pathologic state can cause excessive phosphorylation of a variety of intracellular proteins, causing either complete inactivation, or yielding a toxic gain-of-function effect that could propagate apoptotic pathways. One deleterious example in AD brain is the hyperphosphorylation of the microtubule-associated protein, tau. Routine phosphorylation of tau is necessary to its function in the control of microtubule assembly and stability, as well as intracellular axonal transport [131–133]; however, hyperphosphorylation of tau results in the formation of toxic NFTs, intracellular deposits of hyperphosphorylated tau, involved in the pathogenesis of AD, as well as other tauopathies [134–138]. Therefore, phosphorylation of or by GAPDH during apoptosis could be either a protective mechanism or impart a toxic gain-of-function to, or elicited by, GAPDH that enhances apoptosis in AD brain. Unfortunately, the role(s) of GAPDH phosphotransferase/kinase activity in normal and/or pathological cell function has yet to be made clear.

A final way inhibition of GAPDH activity can contribute to apoptosis is through the glycolytic generation of toxic side-products. GAPDH can undergo many different oxidative modifications, influencing its structure and activity in a variety of neurodegenerative diseases, but especially in AD. Normally, GAPDH catalyzes the reversible phosphorylation of G3P to BPG during glycolysis, while reducing its cofactor NAD^+ to NADH. However, when GAPDH glycolytic activity is impeded, the triose phosphate isomerase and/or aldolase intermediates, dihydroxyacetone phosphate (DHAP) and G3P, begin to accumulate and give rise to the deleterious breakdown product, methylglyoxal (MG). MG is a highly reactive α-ketoaldehyde that readily oxidizes proteins, lipids, and other cellular components, leading to cytotoxicity [139]. Moreover, MG binds to Cys, Lys, and His residues by Michael addition at a faster kinetic rate than does the lipid peroxidation product, HNE [140], although MG, HNE, and acrolein can all alter protein conformation and function [141,142]. A previous study of human red blood cells, in conjunction with type-1 diabetes, revealed the formation of MG after GAPDH inhibition, while *in vivo* administration of exogenous MG was shown to cause renal damage in mice [143]. Moreover, MG is also an isomer of the lipid peroxidation product malondialdehyde (MDA), another highly reactive aldehyde known to accumulate and promote oxidative stress and damage in mouse-models

of accelerated aging, as well as AD brain [140,144, 145]. Considering formation of MG has been shown to result from redox imbalance and perturbation of cellular NAD^+/NADH ratios [146], it is reasonable to believe that MG would play a similar role in AD-related neurodegeneration as MDA.

In a study by Ahmed and colleagues [147] of cerebrospinal fluid (CSF) of 32 AD patients, it was found that the concentration of MG-derived hydroimidazolone (MG-H1) was increased 30-fold compared to age-matched controls. Correlative regression analysis indicated that increased amounts of free MG-H1 adducts were most likely due to oxidative inhibition of GAPDH glycolytic activity, as well as accumulation of triosephospahte in non-vascular compartments and neurons [147]. Moreover, a study by Kuhla et al. [148] examining the ability of reactive carbonyl compounds acrolein, glyoxal, MG, and MDA to induce and/or accelerate tau oligomerization, formation of thioflavin T-positive tau aggregates, and formation of paired helical filaments (PHF), further confirms MG involvement in AD pathology. In this study, MG was recognized as the second most reactive compound amongst the four analyzed, and was able to induce dimer, trimer, and tetramer formation (PHF-like) of synthetic tau filaments in a concentration-dependant manner. It was postulated that the mechanism of MG action on synthetic filaments was through carbon-carbon cross-linking of Lys, Arg, and Cys residues [148].

GAPDH and GSH

The interaction of GAPDH with GSH under normal and pathological conditions is better understood. GSH recognizes the Rossmann fold of GAPDH, and binds to active-site Cys residues, forming disulfide bonds [28]. This S-glutathionylation was first observed as a function of oxidative stress induced by H_2O_2 in human umbilical vein endothelial cells [34], and could be inhibited by both ATP and NAD^+ [28]. S-glutathionylation by GSH changes the pI of GAPDH from 8.1 to 6.9, making the protein more acidic [120], a particular characteristic of those GAPDH isoforms that are often implicated in apoptotic processes [120]. Notably, GAPDH is found to be oxidized in AD brain [28,34,48–55, 149], and regional analysis shows that GAPDH is S-glutathionylated in the inferior parietal lobule (IPL) region of AD brain [48]. Though the role of S-glutathionylation is AD pathology is not yet completely understood, some studies suggest it is a mechanism to protect proteins, like GAPDH, against permanent damage resulting from oxidation of Cys residues to cysteic and cystinic acids by the oxidizing environment of the AD brain [150–152]. This protection helps maintain the redox status of the cellular environment [150–152].

Although, S-glutathionylation, among other oxidative modifications, would inhibit glycolytic activity of GAPDH [48], it would also cause the cell to shift its dependence on glycolysis to the pentose phosphate shunt to augment NADPH production. NADPH is a key cofactor used by GSH-reductase to reduce oxidized glutathione (GSSG), accelerating the cell's antioxidant response elicited by recycling GSH. Therefore, it can be inferred that, under oxidative conditions, GAPDH can act as a switch to redirect glucose metabolism to more appropriate defensive pathways [30]. A momentary deviation from glucose metabolism in such cases would be beneficial, as this diversion provides a protective antioxidant response against ROS and RNS. Unfortunately, due to the unrelenting pathology of AD, the inhibition of glycolysis becomes permanent, and antioxidant defense mechanisms are ultimately overcome by the ever-increasing amount of ROS and RNS generated in AD brain, eliminating the possibility of cell survival [153].

GAPDH and p53

Like GAPDH, p53 is also a multifunctional protein, known largely for its role in DNA transcription and repair [154,155], as well as for its various roles in programmed cell death [156,157]. In particular, results from *in vivo* and *in vitro* studies have shown elevated levels of p53 in neurons related to AD [158–162]. Similarly, studies from our laboratory reveal that p53 levels are significantly higher within the IPL region of AD patients, of which, monomeric and dimeric p53 isoforms were the most abundant [163,164]. Our studies show that p53 is a target of protein oxidation, nitration, and lipid peroxidation within advanced stages of AD neurodegeneration [163,164]. Additionally, monomeric and dimeric p53 isoforms were found to be S-glutathionylated to a higher degree than tetrameric isoforms, which may also illustrate one neuronal defensive mechanism against oxidative stress-induced apoptosis in AD brain [165]. Therefore, the oxidative modification of p53 in AD brain can elicit a toxic gain-of-function to p53 activity that most likely mediates p53-induced apoptotic pathways in AD [163], that are usually associated with two B-cell lymphoma-2 (Bcl-2) family proteins, pro-apoptotic Bax and anti-apoptotic Bcl-2 [166].

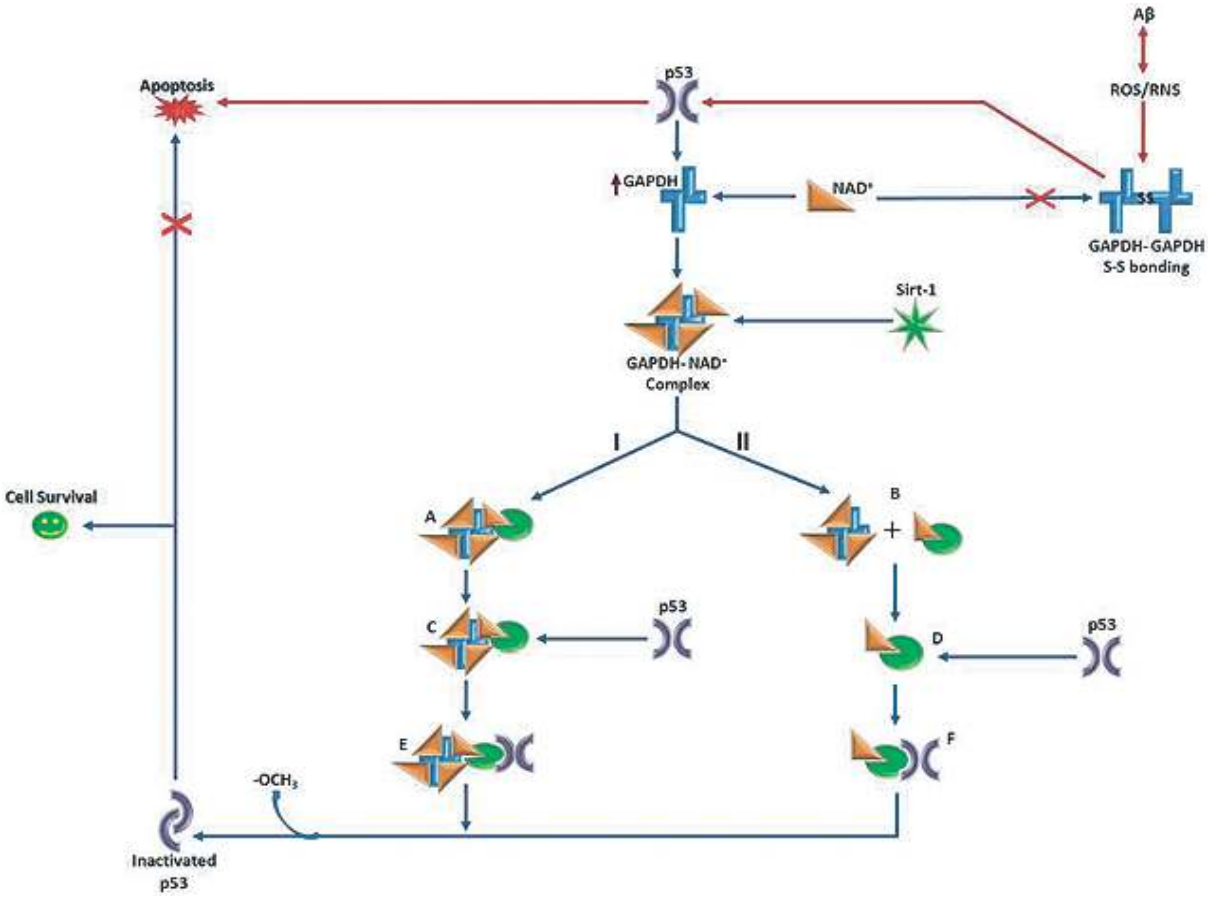

Fig. 6. *p53 and GAPDH interactions*. This diagram represents possible feedback-loop mechanisms of p53 modulation by GAPDH and the NAD^+-dependant protein deacetylase, Sirt-1. Upon binding the GAPDH-NAD^+ complex, Sirt-1 undergoes a conformational change that enables it to bind and remove acetyl groups ($-OCH_3$) from C-terminal Lys residues of p53, inactivating it. Inactivated p53 is unable to induce apoptotic processes; thus, this pathway could lead to cell survival (path shown in blue). At present, the nature of the interaction between Sirt-1 and GAPDH unknown; therefore in this diagram, two possibilities are indicated: I, indicates formation of a GAPDH-NAD^+-Sirt-1 complex (A), while II, indicates NAD^+ transfer from GAPDH, leading to NAD^+-Sirt-1 complex formation (B). In either case, the NAD^+-Sirt-1 interaction likely changes the conformation of Sirt-1 (C and D), which may further facilitate Sirt-1 interaction with p53 to form complexes E and/or F. Additionaly, oxidative stress in AD causes GAPDH to undergo intermolecular disulfide bonding, inhibiting its activity and ability to bind NAD^+, as well as further hindering Sirt-1-induced inactivation of pro-apoptotic p53 (path shown in red).

During an investigation into the role of GAPDH in AraC-induced apoptosis, it was found that transfection of antisense-p53 mRNA into cell culture reduced p53, Bax, and GAPDH mRNA [31]. Moreover, neurons prepared from p53-deficient mice were resistant to AraC-induced apoptosis, in addition to suppressed GAPDH mRNA expression [31], while introducing p53 increased GAPDH expression and triggered apoptosis [29–31]. Other studies indicate that Bcl-2 may regulate the nuclear translocation of GAPDH, thus, protecting cells from apoptosis [29,30,167]. For example, in GT1-7 hypothalamic neurosecretory cells, GAPDH over-expression, nuclear translocation, and subsequent apoptosis were blocked by Bcl-2 over-expression alone [30,31]. Interestingly, Bcl-2 had no effect on the translocation of a GAPDH-GFP conjugate, indicating a unique degree of complexity in the GAPDH translocation mechanism [168]. However, observations with neuroblastoma cells during 6-hydroxydopamine treatment revealed that GAPDH translocates to the Golgi prior to localizing within the nucleus [168]. Taking the above observations into account, it can be inferred that GAPDH is positively regulated by p53; we speculate that p53 can directly induce GAPDH over-expression and nuclear translocation, initiating apoptotic pathways that can be blocked by Bcl-2 in AD brain (Fig. 6).

However, regulation of GAPDH by p53 may not always be to the detriment of the cell. Some studies speculate that induction of GAPDH over-expression by p53 may be an anti-apoptotic mechanism, wherein GAPDH provides an NAD^+ to the p53 inhibitor protein, Sirt-1, in an effort to prevent apoptosis [169]. Sirt-1 is an NAD^+-dependant histone deacetylase protein that removes acetyl groups from a Lys residue present on the C-terminal of p53 [170]. p53 inhibition can increase cellular resistance to stress stimuli, thereby improving cell survival rates [169]. Therefore, regulation of GAPDH expression by p53 could represent a feedback-loop, in which p53-induced over-expression of GAPDH increases the supply of NAD^+ to Sirt-1 that, in turn, inhibits excessive p53-related apoptotic activity (Fig. 6). Moreover, the oxidative modification of GAPDH in AD would decrease its affinity for NAD^+, which would preclude NAD^+ transfer to Sirt-1, resulting in unchecked p53 activity and apoptosis [30, 169]. Given the potentially diverse results from interactions of p53 and GAPDH, additional studies of the roles and interactions of p53 and GAPDH in AD brain are warranted.

GAPDH and NO

NO is a gaseous signaling molecule that plays many critical roles in the central nervous system associated with cognitive function, synaptic plasticity, hormone secretion, and neurotransmission, among others [171, 172]. However, as with many signaling molecules, NO exhibits a duality of function, wherein excess NO can become cytotoxic [173–175]. Furthermore, NO becomes increasingly redox reactive in cells undergoing oxidative stress, a predominant feature in AD brain, by forming toxic RNS and inducing widespread nitrosative stress [172,174,175]. There has been much research indicating a direct interaction between NO and GAPDH by reversible *S*-nitrosylation of critical active-site Cys residues (mainly Cys-149), which inhibits GAPDH dehydrogenase activity [176–178]. Many studies have demonstrated that NO can induce a post-translational modification specific to GAPDH through non-enzymatic, covalent NAD^+ modification [177, 179–181] not observed among other dehydrogenases [180]. Modification of NAD^+ is achieved through auto-ADP-ribosylation of cytosolic GAPDH, in which the adenosine 5'-diphosphoribose (ADP-ribose) moiety of NAD^+ is transferred to another protein [182]. The NO induced *S*-nitrosylation of active-site Cys-149 stimulates mono-ADP-ribosylation of tetrameric GAPDH [181,183], by providing a critical SH-group in close proximity to the NAD^+-binding site [179]. Unfortunately, the role(s) of ribosylated GAPDH in normal and/or pathological cell function have not been described beyond inhibition of enzymatic activity at this point in time. Nevertheless, GAPDH is considered a sensor for NO and nitrosative stress [173,175].

Nitrosative stress is a common phenomenon in neurodegenerative disorders, especially in AD, in which 3-nitrotyrosine (3-NT) formation has been well-documented. Under pathological conditions, NO is produced in excess by one of three NO synthase (NOS) isoforms in the brain, as a pro-inflammatory response [173,174]. Interestingly, although *S*-nitrosylation by NO inhibits enzymatic activity of most proteins, such enzymatic inhibition is not always injurious to the cell. For example, NO can nitrosylate catalytic Cys residues of caspase-3, thereby preventing an apoptotic process [184,185]. However, the consequences of GAPDH NO-modification are not as favorable. In addition to inhibiting its dehydrogenase activity, *S*-nitrosylation of GAPDH confers upon it the ability to bind the E3 ubiquitin ligase, Siah-1 [2,32,33]. Siah-1 is widely expressed in the brain [186,187], and, by itself, can initiate apoptosis by translocating to the nucleus, acetylating, ubiquitinating, and degrading a variety of nuclear proteins, including p53, that mediate cell death [33,188–190].

However, previous studies show that transfection of apoptotic HEK293 cells with GAPDH amplifies nuclear levels of Siah-1 [188], suggesting the stabilized GAPDH-Siah-1 complex is not readily degraded by the ubiquitin proteasome system, thus augmenting GAPDH-Siah-1 nuclear translocation and apoptotic cell death [188]. Moreover, our laboratory has shown a significant increase in GAPDH expression and nitrosylation in AD [51], suggesting that AD pathology creates a synergistic environment augmenting apoptosis induced, in part, by the NO/GAPDH/Siah-1 apoptotic cell death cascade. Consistent with these considerations, a recent study by Sen and coworkers [190] demonstrated that inhibition of GAPDH S-nitrosylation prevented GAPDH-Siah-1 interactions, thereby preventing the initiation of apoptotic processes. These authors also describe a neuroprotective cytosolic protein, GOSPEL ($\sim$52 kDa), that competitively binds cytosolic GAPDH and prevents its nuclear translocation [190]. Interestingly, they found that *S*-nitrosylation of GOSPEL promotes GAPDH binding, thereby enhancing its neuroprotective capabilities [190]. Furthermore, this study indicated that GOSPEL may be a useful approach to

modulate neurodegeneration in AD, Huntington's disease, and other neurodegenerative conditions in which GAPDH may play a critical role.

Hypometabolism, GAPDH membrane binding, and Ca^{2+} flux

A well-known aspect of AD pathology is extensive glucose hypometabolism in concert with hypoxia [191], as the oxidative modification and subsequent inactivation of glycolytic enzymes, such as GAPDH, results in a substantial decline in energy and oxygen bioavailability [191]. Interestingly, many studies have demonstrated that key glycolytic enzymes, such as GAPDH, are electrostatically bound to cell membranes, as well as endoplasmic and sarcoplasmic membranes, of many cell types [25,192–195]. In fact, GAPDH glycolytic activity is directly affected by its interaction with these membranes [194,196–198]. Studies by Galli et al. [194] show that *S*-nitrosylation of active-site thiol groups precludes GAPDH membrane binding, in addition to inhibiting enzyme activity. Therefore, one way the cell regulates glucose metabolism is through GAPDH membrane binding, which prevents modification of active-site Cys residues that are essential to glycolytic activity [194]. A study by Brorson and colleagues [199] involving NO and neuronal energy production demonstrated that an acute depletion of ATP production by high levels of NO was most likely due to inhibition of both mitochondrial respiration and glycolysis, a favored target being GAPDH. Their experiments also imply that the NO-threshold which inhibits glycolytic ATP production is greater than that which inhibits ATP generated by the electron transport chain (ETC) [199]. Inhibition of GAPDH would cause the cell to shift its reliance on glycolysis to the pentose phosphate shunt, which produces NADPH in lieu of NADH. This metabolic switch during oxidative stress can be beneficial as antioxidant enzymes such as glutaredoxin, thioredoxin, and GSH-reductase require NADPH. However, since these anti-oxidant defense mechanisms are eventually overcome in the progression of AD, this switch could permanently uncouple the production of ATP and pyruvate from glycolysis [30,200], thereby contributing to the growing anaerobic environment found in AD brain [194]. Therefore, these experiments imply, in part, a cellular preference for maintenance of glycolytic function over oxidative phosphorylation.

Even though neither glycolysis nor oxidative phosphorylation alone is capable of sustaining cellular energy deficits, the primary source of ATP energy for membrane ion pumps, such as the Na^+/K^+-ATPase and Ca^{2+}-ATPase, is glycolytic [191,195,201]. Previous studies have shown that GAPDH binds to the IP3 receptor and SERCA pump [24,25], thereby coupling glycolysis and ion pump activity [195,202], which would account for the cell's glycolytic ATP preference [195, 203]. In a study by Kahlert et al. [202], glycolytic ATP was shown to be more important for maintaining ER Ca^{2+} stores and IP3-mediated Ca^{2+} signaling than the ETC, as the addition of iodoacetic acid (IA; non-specific SH-group modifier) together with exogenous pyruvate to cultured astrocytes could not restore intracellular ATP and basal Ca^{2+} levels, nor membrane potential [201,202]. IP3R-bound GAPDH modulates Ca^{2+} release via NADH production [24]; therefore, NO modification (like IA) of critical GAPDH active-site Cys residues under pathological conditions would not only cause a metabolic shift from glycolysis to the pentose phosphate pathway, but also trigger the cytoplasmic release of ER Ca^{2+} stores, leading to Ca^{2+} excitotoxicity, predominant in AD pathology [204–206]. Therefore, maintenance of glycolytic GAPDH function is essential not only to ATP and pyruvate production, but also to the maintenance of intracellular Ca^{2+} levels and prevention of membrane depolarization that could lead to cell death [191], especially in AD brain.

GAPDH, AβPP, Aβ, and tau

A systematic meta-analysis of AD genetic association study by Bertram and coworkers [207] showed that 13 genes play a significant role in development of AD, one of which, was GAPDH. Moreover, a study by Wang et al. [208] reported the conversion of GAPDH to a detergent-insoluble state was coupled with AD progression. These analyses, in addition to others, serve to emphasize the critical role GAPDH plays in neurodegeneration, as the GAPDH gene (*GAPD*) and pseudogenes [57–61] have been suggested to be risk factors in late-onset AD [209,210]. Initial investigations into the involvement of GAPDH in AD reported that a $\sim$ 38 kDa protein, identified as GAPDH, was consistently increased during age-induced apoptosis [211]. Further studies demonstrated cross-reactivity between GAPDH and the monoclonal antibody Am-3, which recognizes AβPP but not Aβ, raised against amyloid plaques extracted from the brain of AD patients [212]. However, Tamaoka and colleagues [213] suggested that such cross-reactivity was not a result of similar homology between epitopes, since they would lie outside

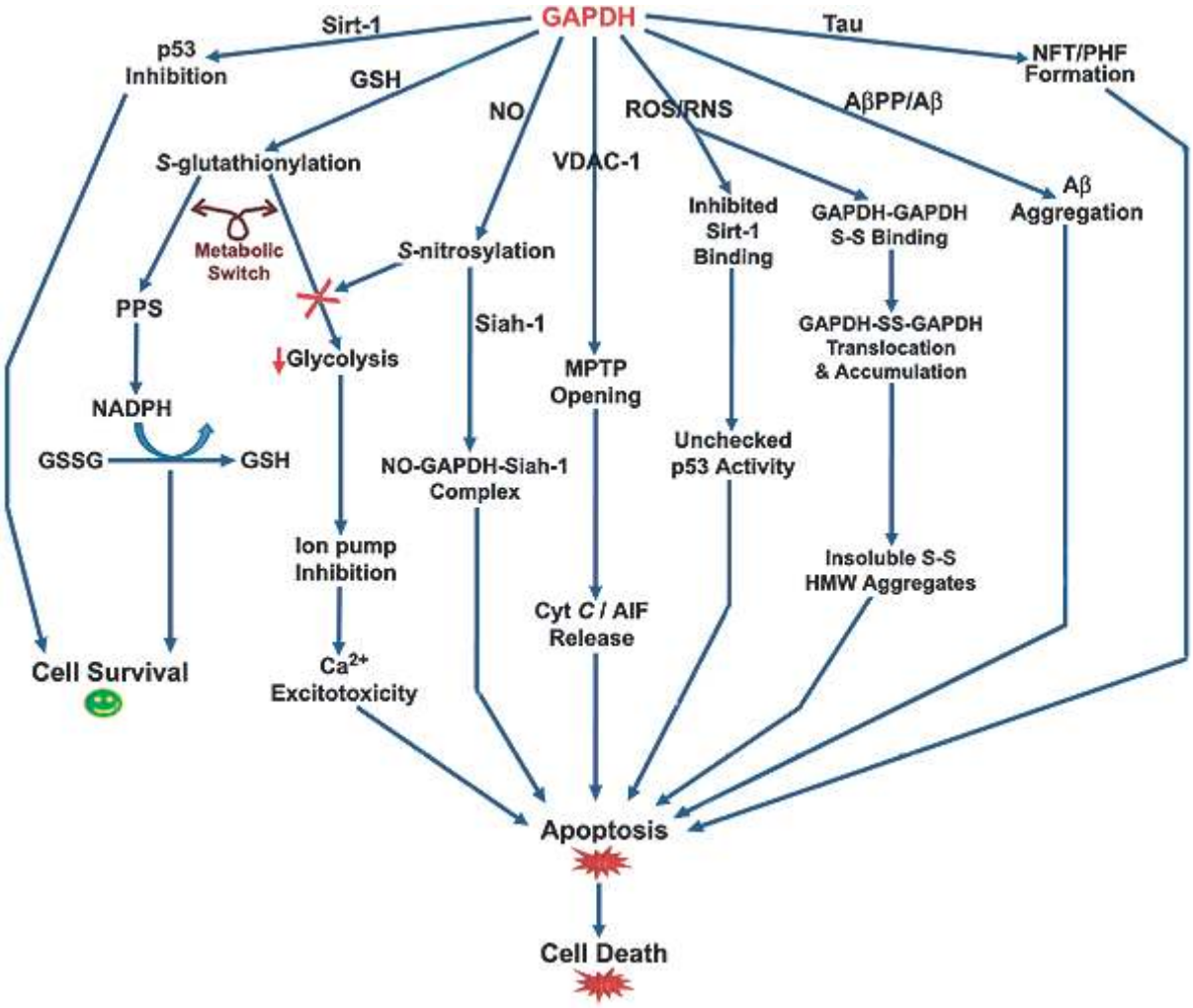

Fig. 7. *GAPDH functional diversity in AD brain*. This arrow diagram is a summary of the various indirect and direct signaling pathways, pro- and/or anti-apoptotic, that GAPDH triggers upon association with various proteins in AD brain. Abbreviations: GSH, gluthathione; GSSG, oxidized GSH; PPS, pentose phosphate shunt; NO, nitric oxide; MPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species; RNS, reactive nitrogen species; S-S, disulfide bond; HMW, high molecular weight; NFT, neurofibrillary tangles; PHF, paired helical filaments. See text.

the Aβ_{1-42} sequence region on AβPP [213–218], but were most likely due to conformational similarities between Aβ and GAPDH. Furthermore, a direct interaction between GAPDH and AβPP has been reported, in which rat brain monomeric GAPDH interacted with the cytoplasmic C-terminal domain of recombinant AβPP, while maintaining its glycolytic activity [36]. Later studies confirmed that brain-derived GAPDH also could bind a variety of Aβ isoforms, displaying greatest affinity for Aβ_{1-42} [101,102,219].

Indeed, GAPDH is often identified as a major component of amyloid plaques and even NFTs in AD brain [102,208]; however, some researchers have questioned whether or not its presence indicates a direct role in Aβ aggregation and/or NFT formation. Recent research suggests the manner in which GAPDH accumulates in amyloid plaques is not due to a high concentration in neurons, or the binding of GAPDH to pre-aggregated Aβ; rather, these studies demonstrate that only oxidized and denatured forms of GAPDH (i.e., monomeric, dimeric, or unfolded polypeptide chains) were able to form highly stable complexes with Aβ [102,219]. Moreover, these non-native GAPDH isoforms only could bind soluble Aβ species, as opposed to pre-aggregated structures, indicating the direct involvement of GAPDH in amyloid aggregation. Interestingly, it also was suggested that Aβ could potentially accelerate [thermo] inactivation of native GAPDH,

as the interaction between Aβ and partially unfolded GAPDH species would shift the equilibrium to favor denaturation [219]. Likewise, a study by Cumming and Schubert [220] showed that Aβ promotes GAPDH disulfide binding, suggesting that oxidative stress induced by Aβ neurotoxicity not only increases levels of denatured GAPDH, but also promotes its nuclear translocation and pro-apoptotic action.

Research on NFT formation in AD brain reports results similar to those of GAPDH and Aβ/AβPP. As mentioned above, hyperphosphorylation of the microtubule-associated protein tau is rampant in AD brain, leading to formation of neurotoxic NFTs, of which, PHF-tau is a major component [136,137, 221]. Previous LC-MS/MS analysis demonstrated that GAPDH co-localized with NFTs and immunoprecipitated with PHF-tau from the temporal cortex of AD brain [208,222]. Further studies by Chen et al. [223], showed that tau was able to bind and promote the denaturation and inactivation of GAPDH *in vitro*. However, their research also established that phosphorylated and pre-aggregated PHF-tau were unable to bind or affect GAPDH denaturation or activity [223], suggesting a direct involvement of GAPDH in tau aggregation and NFT formation in AD brain. Although, the above-mentioned research reveals the critical role GAPDH plays in both Aβ and tau aggregation, how these GAPDH-Aβ, -AβPP, and -tau complexes affect other cellular processes in AD brain remains to be determined. However, it is clear that maintenance of GAPDH form and function is integral to the prevention of protein aggregation pathology in AD brain.

CONCLUSION

It is clear that GAPDH possesses a myriad of functions in addition to basic glycolysis, and the purpose of those functions in normal and pathologic cellular environments remains a topic of great interest. Based on the research described above, there is strong evidence for direct and indirect involvement of GAPDH in AD pathology (Fig. 7). Decreased GAPDH glycolytic activity, in addition to oxidative and post-translational modifications, like S-gluatathionylation and S-nitrosylation, are distinct markers of cellular stress in AD pathology that significantly impact intracellular homeostasis by promoting GAPDH-induced Ca^{2+} excitotoxicity through IP3R and SERCA binding, as well as interaction with pro-apoptotic proteins such as p53, Siah-1, and the MPTP through VDAC-1 binding. These indirect apoptotic insults contribute to a host of feedback-loop mechanisms, in which AD pathology is able to perpetuate an ongoing cycle of cell death that ultimately overcomes inherent antioxidant defenses. Although S-glutathionylation and S-nitrosylation of GAPDH may initially act as a metabolic switch to protect the cell, in AD brain these modifications eventually inhibit GAPDH activity and glucose metabolism indefinitely.

The direct involvement of GAPDH in AD is somewhat more intriguing, as its association with AβPP, Aβ_{1-40}/Aβ_{1-42}, and tau demonstrates its direct participation in the aggregation of these three species into their insoluble counterparts. However, it should be noted that in order for GAPDH to partake in amyloid or tau aggregation, it must first succumb to denaturation and/or oxidative modification, demonstrating that GAPDH inhibition and pro-apoptotic function is a result of ongoing oxidative stress and damage that is itself but a secondary effect of an as of yet unknown primary scaffold of AD pathology. Although the exact mechanisms of many of the GAPDH interactions and processes described in this review are not yet clear, one can be certain that the structure and subcellular localization of GAPDH is vital to understanding the many roles it plays in normal and pathological cell function. Therefore, it seems that maintenance of GAPDH structure and activity may be a promising therapeutic target to slow or halt neurodegeneration in AD brain.

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