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REVIEW

Multifunctional roles of enolase in Alzheimer's disease brain: beyond altered glucose metabolism

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Abstract

Enolase enzymes are abundantly expressed, cytosolic carbon-oxygen lyases known for their role in glucose metabolism. Recently, enolase has been shown to possess a variety of different regulatory functions, beyond glycolysis and gluconeogenesis, associated with hypoxia, ischemia, and Alzheimer's disease (AD). AD is an age-associated neurodegenerative disorder characterized pathologically by elevated oxidative stress and subsequent damage to proteins, lipids, and nucleic acids, appearance of neurofibrillary tangles and senile plaques, and loss of synapse and neuronal cells. It is unclear if development of a hypometabolic environment is a consequence of or contributes to AD pathology, as there is not only a significant decline in brain glucose levels in AD, but also there is an increase in proteomics identified oxidatively modified glycolytic enzymes that are rendered inactive, including enolase. Previously, our laboratory identified α-enolase as one the most frequently up-regulated and oxidatively modified proteins in amnestic mild cognitive impairment (MCI), early-onset AD, and AD. However, the glycolytic conversion of 2-phosphoglycerate to phosphoenolpyruvate catalyzed by enolase does not directly produce ATP or NADH; therefore it is surprising that, among all glycolytic enzymes, α-enolase was one of only two glycolytic enzymes consistently up-regulated from MCI to AD. These findings suggest enolase is involved with more than glucose metabolism in AD brain, but may possess other functions, normally necessary to preserve brain function. This review examines potential altered function(s) of brain enolase in MCI, early-onset AD, and AD, alterations that may contribute to the biochemical, pathological, clinical characteristics, and progression of this dementing disorder.

Keywords: Alzheimer's disease, amyloid β-peptide, *c-Myc*, enolase, MAPK/ERK1/2, plasmin plasminogen.

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Enolase enzymes (EC 4.2.1.11) are a superfamily of abundantly expressed carbon-oxygen lyases known for their role in glycolysis and gluconeogenesis. Glycolytic enzymes, like enolase, are among some of the most well-characterized proteins to date; yet, enolase isoforms were previously believed to perform exclusively 'house-keeping' functions for the cell. However, recent studies have demonstrated that enolase possesses a variety of different regulatory properties, in addition to their glycolytic functions in the brain (Pancholi 2001). In particular, enolase has been reported to be a neurotrophic factor, 14-3-2 (Takei *et al.* 1991; Hattori *et al.* 1994, 1995), a hypoxic stress protein (Aaronson *et al.* 1995), *c-Myc* binding protein and transcription factor (Ray and Miller 1991; Subramanian and Miller 2000), and a strong plasminogen (PGn) binding protein (Nakajima *et al.* 1994; Pancholi and Fischetti 1998). Furthermore, many of these non-glycolytic functions have been implicated in hypoxia

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Abbreviations used: AD, Alzheimer's disease; ALS, amyolateral sclerosis; AβPP, amyloid-β precursor protein; ChoRE, carbohydrate response element; DLB, dementia with Lewy bodies; EOAD, early-onset AD; ERK1/2, extracellular signal-regulated kinase 1/2; FAD, familial AD; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HD, Huntington's disease; HIF-1α, hypoxia-inducible factor-1α; HNE, 4-hydroxy-2-nonenal; KPI, Kunitz-type serine protease inhibitor; L-LTP, late-phase long term potentiation; LRP-1, lipoprotein-related receptor-1; MAPK, mitogen-activated protein kinase; MBP-1, *c-Myc* binding protein-1; MCI, mild cognitive impairment; PD, Parkinson's disease; PGn, plasminogen; ROS, reactive oxygen species; tPA, tissue-PGn activator; uPA, urokinase-PGn activator.

and ischemia (Mizukami *et al.* 2004; Sousa *et al.* 2005), as well as Alzheimer's disease (AD) (Parnetti *et al.* 1995; Dotti *et al.* 2004). [See Supporting Information on enolase structure, active site, reactions and isoform choromosomal and tissue localisation in Appendix S1, Table S1 and Figures S1–S4 in the online version of this review.]

A largely sporadic, age-associated neurodegenerative disorder, AD typically affects populations over the age of 60. Pathologically, AD can be characterized by elevated oxidative stress and subsequent damage to brain proteins, lipids, and nucleic acids, the appearance of neurofibrillary tangles and senile plaques, and eventual loss of synapse and neuronal cells that result in a progressive decline in cognitive function. In rare instances, autosomal dominant mutations in the *amyloid*-β *precursor protein* (*AβPP*) or *presenilin* genes 1 and 2 (*PS-1/-2*) cause a familial form of AD (FAD) that produces the same clinical and pathological consequences as sporadic AD, but at a much earlier age (~30 years old) (Citron *et al.* 1992; Scheuner *et al.* 1996; Sturchler-Pierrat *et al.* 1997; Wisniewski *et al.* 1998). Recently, α-enolase has been identified as one of the most frequently identified differentially expressed brain proteins in human and animal tissues (Petrak *et al.* 2008). As described below, previous studies by our laboratory have found α-enolase to be one of the most consistently up-regulated and oxidatively modified proteins in brain of subjects with early-onset AD (EOAD), AD, and amnestic mild cognitive impairment (MCI) (Butterfield and Sultana 2007; Butterfield *et al.* 2007), arguably the earliest form of AD (Petersen *et al.* 1999; Winblad *et al.* 2004). These findings suggest that enolase may possess one or more additional functions, beyond simple glucose metabolism, that could be integral to both normal and pathological brain function. Therefore, the intent of this review is to examine potential function(s) of α- and γ-enolase isoforms in AD brain.

Enolase functional diversity

Beyond glucose metabolism (Fig. 1), enolase enzymes have been reported to have a number of other non-glycolytic functions, such as the ability to bind polynucleotides (al-Giery and Brewer 1992), being a τ-crystallin protein (Wistow *et al.* 1988), neurotrophic factor, 14-3-2 (Takei *et al.* 1991; Hattori *et al.* 1994, 1995), heat-shock protein 48 (HSP48) (Iida and Yahara 1985), hypoxic-stress protein (Aaronson *et al.* 1995), *c-Myc* binding and transcription protein (Ray and Miller 1991; Subramanian and Miller 2000), and a strong PGn binding protein (Nakajima *et al.* 1994; Pancholi and Fischetti 1998), among others (Table 1). This wide array of functions can be attributed to different DNA base sequences within enolase genes. For example, the promoter region of *ENO1* contains a copy of the viral core consensus sequence [GTGG(A/T)(A/T)(A/T)G] (Jones *et al.* 1988), two copies of the octanucleotide sequence (ATTTGCAT) found in immunoglobulin (Ig) gene enhancers and promoters (Jones *et al.* 1988), a C2 binding site (CATGTG) present in Ig heavy chain enhancers (Peterson and Calame 1989), part of the liver-specific enhancer binding site sequence (TCNTACTC) (Grayson *et al.* 1988), a cAMP response element sequence (position – 298) (Grayson *et al.* 1988), and specificity protein 1 (SP1) transcription factor binding site (Briggs *et al.* 1986). Although the functional significance of many of these elements is unknown, other sequences are better-characterized, particularly the carbohydrate response element (ChoRE) motif (^{59}-CACGTG-^{39}). Upon glucose stimulation, transcription factors, such as hypoxia-inducible factor-1α (HIF-1α) and *c-Myc*, bind the *ENO1* ChoRE motif and initiate transcription of the enolase enzyme (Thompson and Towle 1991; Towle 1995; Dang 1999).

Enolase and *c-Myc*

Typically, functional diversity of proteins originates during transcriptional regulation. For example, Giallongo, *et al.* (Giallongo *et al.* 1990) discovered multiple transcription start sites in *ENO1* that are consistent with the lack of a canonical TATA box 19–27 base-pairs upstream of its cap-site, which is primarily responsible for accurately positioning the correct mRNA start site. The significance of this finding is illustrated in its effects on translation initiation, which usually occurs at the first in-frame, 5′-AUG codon, representing the optimal context (Kozak 1999). However, reinitiation, direct internal initiation, and leaky scanning caused, in part, by the lack of a start site-directing TATA box, can produce more than one protein from a single mRNA, such as in the case of *ENO1* (Giallongo *et al.* 1990; Kozak 1999; Feo *et al.* 2000). Thus, the two *ENO1* gene products, α-enolase (48 kDa) and *c-Myc* binding protein-1 (MBP-1; ~37 kDa), share 97% sequence similarity (Giallongo *et al.* 1986). Two single-base insertions in MBP-1 result in a reading frame shift affecting its N-terminus as compared to the α-enolase coding region, while the C-terminal regions of both enzymes are identical (Ray and Miller 1991; Onyango *et al.* 1998). Furthermore, α-enolase and MBP-1 have similar function, although quite different subcellular fates.

Normally, α-enolase is directed to the cytoplasm, in which it carries out its metabolic role, while MBP-1 is located in the nucleus where it is involved in transcriptional regulation of the *c-Myc* protooncogene. *c-Myc* is a DNA-binding phosphoprotein critical in the control of cell proliferation, differentiation, and apoptosis (Spencer and Groudine 1991; Evan *et al.* 1992; Marcu *et al.* 1992) that is commonly over-expressed in tumor cells. Like most 'housekeeping' genes, *ENO1* mRNA translation is primarily under developmental control, significantly up-regulated during cellular growth and practically undetectable during quiescent phases (Holland *et al.* 1983; Giallongo *et al.* 1990). In transformed cells, the over-expression of *c-Myc* stimulates abnormal cell proliferation and up-regulation of several glycolytic enzymes, including α-enolase, in order to accommodate the mounting

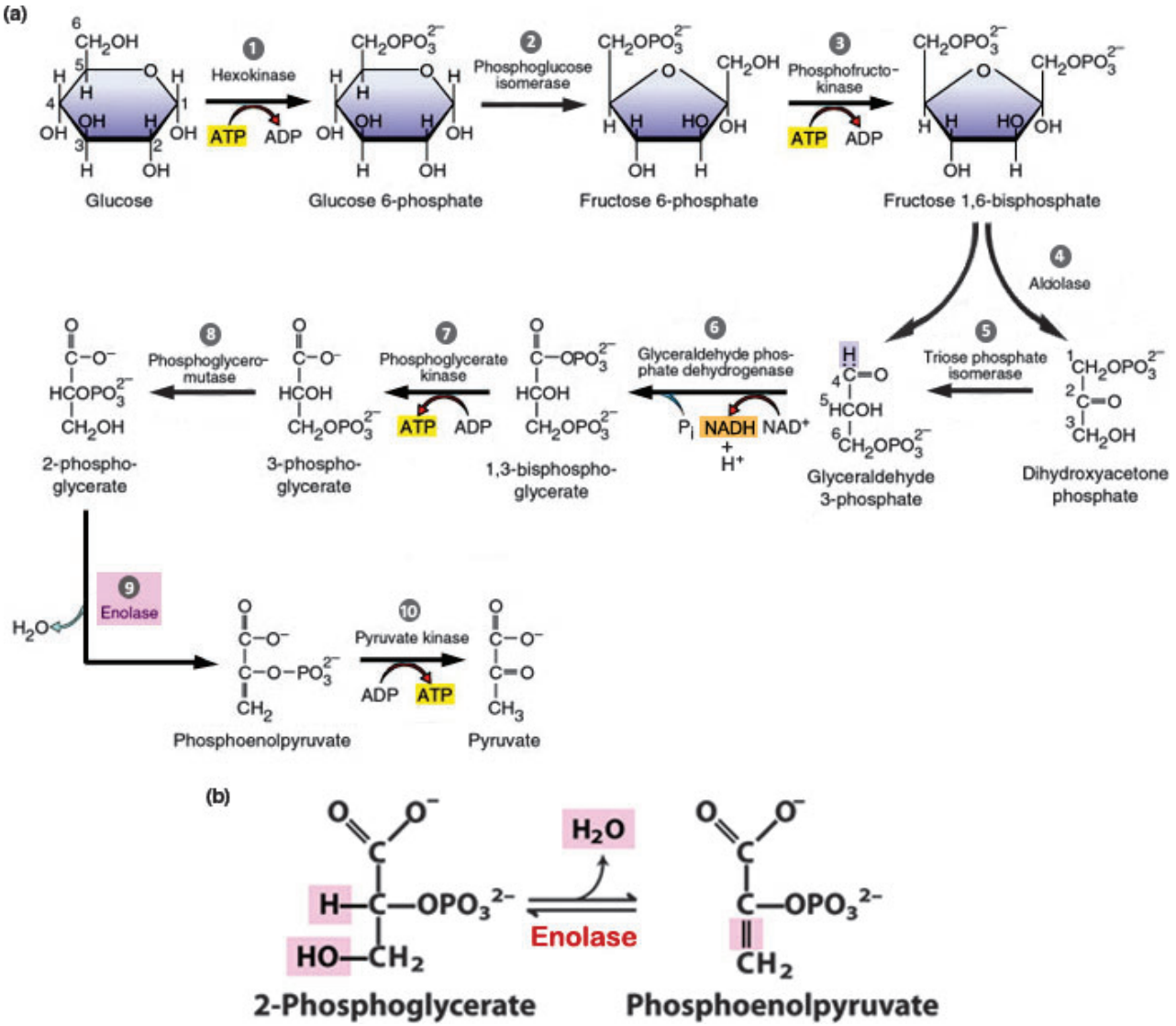

Fig. 1 (a) Glycolysis. Schematic representation of aerobic glycolysis. Because two 3-carbon triose chains are produced from the reaction between aldolase and fructose-1,6-bisphosphate, steps 5–10 are completed twice (not shown). ATP production in steps 7 and 10 is thought to be the chief fuel-source for plasma membrane ion pumps, including the Na^+/K^+-ATPase and Ca^{2+}-ATPase, rather than ATP produced by mitochondrial oxidative phosphorylation [adapted from (Karp 2003)]. (b) Enolase reaction. Step 9 in the glycolytic chain involves conversion of 2-phosphoglycerate (2-PGA) to phosphoenolpyruvate (PEP) by enolase [adapted from (Nelson and Cox 2009)].

energy deficit (Osthus *et al.* 2000; Hurlin and Dezfouli 2004; Kim and Dang 2005). In turn, MBP-1 negatively regulates *c-Myc* transcription (Ray and Miller 1991; Chaudhary and Miller 1995; Ray 1995), acting as a tumor suppressor and completing a regulatory 'feedback-loop' of both *c-Myc* and glycolytic activity (Sedoris *et al.* 2007). Interestingly, although MBP-1 does not have enolase enzyme activity, both α-enolase and MBP-1 are able to act as tumor supressors because *c-Myc* down-regulating activity lies within two hydrophobic N- and C-terminal regions present in both *ENO1* translation products (Bentley and Groudine 1986; Subramanian and Miller 2000).

Enolase and the plasminogen system

The PGn system is best known for the pivotal role it plays in maintenance of vascular potency and thrombolysis, by dissolving fibrin (Plow *et al.* 1991; Collen 1999). In order to elicit function, the glycoprotein PGn binds cell surface receptors via kringle domains that recognize exposed C-terminal lysine residues. Interestingly, PGn binds these receptors with low affinity, yet is more readily activated than free PGn (Plow *et al.* 1995), and subsequently produced plasmin has greater enzymatic activity and is protected from inactivation by inhibitors, such as α_2-antiplasmin (Plow *et al.* 1991). Therefore, virtually any surface protein exposing

Table 1 Enolase functional diversity and/or involvement in disease

Functional diversity
Glycolytic/gluconeogenesis enzyme
Eye lens τ-Crystallin protein
Plasminogen binding protein in:
Various human diseases
Group A *Streptococci/Pneumococci* bacterial diseases
c-Myc binding protein (MBP-1) and transcription factor in:
Tumor formation
Metastasis
Tumor marker
Heat-shock protein in yeast
Hypoxic-stress protein
Centrosome component in HeLa cells
Toxin B in *Clostridium difficile* (?)
Immunodominant antigen in:
Invasive *Candidiasis* fungal disease
Candida albicans and *Aspergillus* enolase-specific IgE responses
Anti-centrosome antibody
Anti-neutrophil cytoplasmic antibody (ANCA) in:
Vasculitis
Systematic lupus erythematosus
Discoid lupus erythematosus
Nephritis/primary membranous nephropathy
Inflammatory bowel diseases:
Ulcerative colitis
Crohn's disease
Behçet's disease anti-endothelial antigen
Liver diseases
Primary sclerosing cholangitis
Primary biliary cirrhosis
Autoimmune hepatitis
Polyglandular candidal ectodermal dystrophy
Cancer-associated retinopathy
Endometriosis
Acute rheumatic fever
Post-*Streptococcal* neurological disorder/obsessive-compulsive disorder/Tourette's syndrome (?)
Other roles/disease involvement
Site-specific organization of tubule/centrosome
Streptococcus intermedius/Streptococcus mutans-mediated dental caries (?)
Plasmodium falciparum malaria
Rheumatoid arthritis

(?) signifies unknown/debated enolase functions and/or disease involvement [adapted from (Pancholi 2001)].

C-terminal lysines has the potential to bind and activate PGn processes, in addition to contributing to the high density and broad distribution of the many heterogenous PGn binding sites (Plow *et al.* 1995). Gangliosides (Miles *et al.* 1989), glycosaminoglycans (Andrade-Gordon and Strickland 1986), annexin II (Cesarman *et al.* 1994; Hajjar *et al.* 1994), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Lottenberg *et al.* 1992), and α-enolase (Miles *et al.* 1991; Redlitz *et al.* 1995; Pancholi and Fischetti 1998) are just a few examples of the different types of proteins reported to be PGn surface receptors. Most remarkable, however, is the discovery that glycolytic enzymes, GAPDH and α-enolase, are able to integrate into the cell membrane without possessing a signal sequence and retain enzymatic activity (Pancholi and Fischetti 1998). Some researchers speculate that a hydrophobic domain (^{33}-AAVPSGASTGIY-^{44}) within α-enolase might serve as an internal signal sequence, allowing its integration (von Heijne *et al.* 1991), while others suggest post-translational acylation (Bottalico *et al.* 1993) or phosphorylation (Cooper *et al.* 1984) may be a means of membrane association. Nevertheless, these two cytosolic enzymes are now part of a growing group of proteins that lack signal sequences, but are transported to the cell surface by an unknown mechanism.

However, binding surface receptors, like α-enolase, alone cannot activate PGn conversion to plasmin; the PGn-proteolytic cascade must begin with cleavage by either tissue-PGn activator (tPA) or urokinase-PGn activator (uPA) (Bergman *et al.* 1997), both of which, can be found in human brain (Sappino *et al.* 1993; Carmeliet *et al.* 1994). tPAs and/or uPAs initiate fibrinolysis by binding fibrin aggregates, which leads to a conformational change that dramatically increases their affinity for PGn. As a result, PGn is cleaved by tPA/uPA into proteolytic plasmin (Tucker *et al.* 2000b). Of particular interest in human brain is tPA, a serine protease correlated with hippocampal late-phase long term potentiation (L-LTP) (Kandel 2001; Pang and Lu 2004). Transgenic mice over-expressing tPA exhibit enhanced L-LTP and improved spatial learning (Madani *et al.* 1999). Induction of L-LTP, in turn, enhances neuronal expression of tPA within the hippocampus (Qian *et al.* 1993), perpetuating a cycle of secretion and growth necessary for brain development. In addition, PGn has also been implicated in wound healing and inflammation through its involvement in cell proliferation and migration (Kalderon 1979, 1982; Plow *et al.* 1991; Tarui *et al.* 2002), as well as many intracellular signaling events by activation of proenzymes (Liotta *et al.* 1981; Blasi *et al.* 1987; Nagase *et al.* 1990), prohormones (Virji *et al.* 1980), progrowth factors (Rifkin *et al.* 1990; De Sousa *et al.* 2005), and procytokines (Nakagawa *et al.* 1991; Konakova *et al.* 1998). Thus, in this review, the role enolase plays in conjunction with the tPA/PGn system will be analyzed with respect to subjects with AD.

Enolase and Alzheimer's disease

Alzheimer's disease is an age-associated neurodegenerative disorder that typically affects the elderly, aged 60 and above. However, in rare instances it can affect younger populations, as early as 30 years old, in FAD (Citron *et al.* 1992; Scheuner *et al.* 1996; Sturchler-Pierrat *et al.* 1997; Wisniewski *et al.* 1998). Both AD and FAD can be characterized clinically by a progressive decline in cognitive function, and

pathologically by synapse and neuronal cell loss, as well as the appearance of neurofibrillary tangles and senile plaques. Other hallmarks of AD and FAD pathology are oxidative stress and damage that induce protein and nucleic acid oxidation, lipid peroxidation, and apoptosis, which lead to declining brain function and loss of synapses and neurons (Markesbery 1999; Aksenov *et al.* 2001; Butterfield *et al.* 2001, 2002; Butterfield 2002; Butterfield and Lauderback 2002; Bader Lange *et al.* 2008). In the same way, MCI, considered a transition point between normal cognitive aging and probable AD (Petersen *et al.* 1999; Winblad *et al.* 2004), has also been reported to have elevated oxidative stress levels (Keller *et al.* 2005; Markesbery *et al.* 2005; Butterfield *et al.* 2006b, 2007; Butterfield and Sultana 2007; Lovell and Markesbery 2007; Markesbery and Lovell 2007). Oxidative modification of proteins during disease progression, in turn, results in diminished and/or complete loss of protein function, as indexed by levels of protein carbonyls, 3-nitrotyrosine, protein-/lipid-bound 4-hydroxy-2-nonenal (HNE), and *S*-glutathionylation (Butterfield and Stadtman 1997).

According to a recent report, α-enolase has been identified as differentially expressed in about 30% of all 2D-gel electrophoresis-based experiments in human and animal tissues published in recent issues of *Proteomics* (Petrak *et al.* 2008), rendering it one of the top 15 most frequently identified differentially expressed proteins. Our laboratory has reported that enolase is oxidatively modified in MCI, EOAD, and AD. In these studies compared to control brain, α- and γ-enolase were found to be excessively carbonylated (Castegna *et al.* 2002; Butterfield *et al.* 2006a,b; Sultana *et al.* 2006a), nitrated (Castegna *et al.* 2003; Sultana *et al.* 2006b; Reed *et al.* 2009), HNE-modified (Reed *et al.* 2008; Perluigi *et al.* 2009), and *S*-glutathionylated (Newman *et al.* 2007) in brain areas such as the inferior parietal lobule, hippocampus, and frontal cortex, but not in cerebellum, a brain region essentially devoid of pathology in AD. Whether the extensive oxidative modification of enolase is simply because of its proximity to the many redox reactions occurring throughout the cell or a result of structural susceptibility to oxidation is unknown. However, both possibilities are conceivable as enolase can be found in numerous regions of the cell and possesses many active-site Lys and His residues that are extremely susceptible to Michael addition by compounds such as HNE (Butterfield and Stadtman 1997). For example, γ-enolase has been identified as a component of the NADH-dichlorophenol-indophenol reductase complex, one of several *trans*-plasma membrane oxidoreductases located within synaptic plasma membranes and recycling vesicles (Bulliard *et al.* 1997). *Trans*-plasma membrane oxidoreductases function as anti-oxidant enzymes and extracellular redox sensors that regulate cell proliferation and axonal guidance in response to external pro- or anti-oxidants (Fuhrmann *et al.* 1989; Toole-Simms *et al.* 1991), thereby placing enolase in direct contact with reactive oxygen species (ROS) that could readily modify its activity and structure. Furthermore, our laboratory has also reported that enolase is present within mitochondria, one of the largest ROS producers within the cell, and contributes this organelle's function (Poon *et al.* 2005).

Another predominant feature in MCI, EOAD, and AD is the manifestation of glucose hypometabolism (Mielke *et al.* 1996), associated with the oxidative-inactivation of several glycolytic enzymes, including enolase (Castegna *et al.* 2002; Butterfield *et al.* 2006b; Sultana *et al.* 2007) (Fig. 2). Because the brain is one of the greatest consumers of glucose, hypometabolism can cause the up-regulation of glycolytic enzymes in an effort to combat the mounting energy deficit and hypoxic environment (Mielke *et al.* 1996). Moreover, previous studies have shown that cells resistant to Aβ toxicity had a greater flux of glucose through glycolysis and the hexose monophosphate shunt (Soucek *et al.* 2003). Interestingly, although the glycolytic function of enolase does not directly produce ATP or the reduced energy carrier NADH (Fig. 1), in all studies of MCI, EOAD, and AD brain from our laboratory, enolase levels are increased (Castegna *et al.* 2002; Sultana *et al.* 2007), while levels of pyruvate kinase, phosphoglycerate kinase, and GAPDH vary throughout disease progression (Fig. 2). Furthermore, our laboratory has shown that α-enolase was one of only four proteins, glycolytic or otherwise, consistently up-regulated and oxidatively modified in the progression from MCI to AD (Fig. 2). These remarkable findings suggest that enolase may well be involved in more than just metabolic processing of glucose, but perhaps possesses other critical functions vital to preserving brain function, which are discussed subsequently in this review.

Enolase, the plasminogen system, and glutamate excitotoxicity

Glutamate excitotoxicity, a well-known phenomenon in AD brain, is characterized by the increased release and impaired uptake of glutamate, which mediates the toxic build-up of extracellular glutamate, leading to overstimulation of glutamate receptors, NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate receptors. Collapsing cellular ATP reserves and Na^+ ion gradients exacerbate this process, and eventually lead to rising intracellular Ca^{2+} levels, because of the opening of voltage-gated Ca^{2+} channels and release of Ca^{2+} from the endoplasmic reticulum (Fig. 3). Increased cytoplasmic Ca^{2+}, in turn, further depolarizes the cell membrane and activates cytotoxic intracellular pathways that lead to neuronal death, such as inducing the Ca^{2+}-dependent secretion of the serine protease, tPA (Gualandris *et al.* 1996; Baranes *et al.* 1998; Fernandez-Monreal *et al.* 2004b). During glutamate excitoxicity, excessive neuronal activity and extracellular secretion of tPA can cause neuronal death by augmenting microglial activation

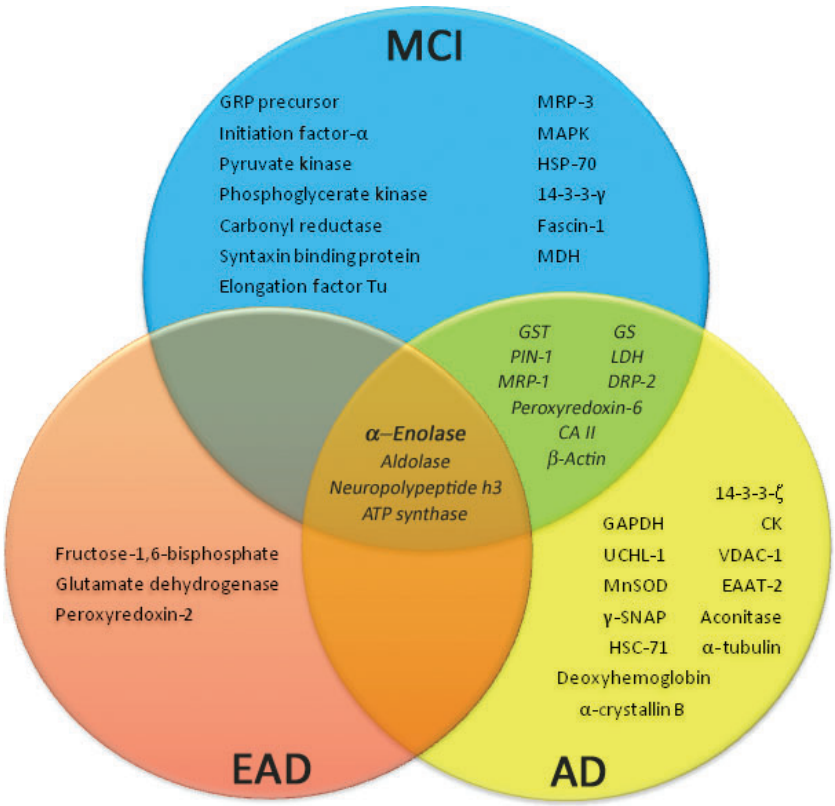

Fig. 2 Oxidatively modified and/or glutathionylated proteins in MCI, EOAD, and AD brain identified by redox proteomics studies from our laboratory (Castegna et al. 2002, 2003; Butterfield et al. 2006a,b; Sultana et al. 2006a,b; Newman et al. 2007; Reed et al. 2008, 2009; Perluigi et al. 2009). This diagram shows the interrelation of all proteins found to be oxidatively modified in MCI, EOAD, and AD brain from our laboratory. GRP precursor, glucose-regulated protein precursor; MRP-1, multidrug-resistant protein-1; MAPK, mitogen-activated protein kinase; HSP70, heat-shock protein-70; MDH, malate dehydrogenase; GST, glutathione *S*-transferase; GS, glutamine synthetase; PIN-1, peptidyl-prolyl *cis*/*trans* isomerase-1 (PPIase); LDH, lactate dehydrogenase; DRP-2, dihydropyrimidinase-related protein-2; CAII, carbonic anhydrase II; HSC-71, heat-shock cognate-71; γ-SNAP, soluble *N*-ethylmaleimide-sensitive factor attachment protein-γ; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; UCHL-1, ubiquitin carboxy-terminal hydrolase L-1; VDAC-1, voltage dependent anion channel-1; CK, creatine kinase; EAAT-2, excitatory amino acid transporter-2; MnSOD, manganese superoxide dismutase.

(Rogove and Tsirka 1998), increasing plasmin activation and degradation of laminin (Tsirka *et al.* 1995; Chen and Strickland 1997), and potentiating NMDA receptor signaling processes (Tsirka *et al.* 1995, 1997; Nicole *et al.* 2001).

Apoptotic cells often secrete and/or exhibit signal molecules within the membrane that allow activated microglia to scavenge, recognize, and bind damaged cells that require clearance, in order to prevent further injury to surrounding areas. In a study by Siao and Tsirka (2002), glutamate-injured neurons were shown to release sufficient tPA to activate tPA−/− microglia in a cytokine-like manner (Rogove and Tsirka 1998). Furthermore, activated microglia were shown to secrete tPA in a proteolytic-independent manner, activating neighboring microglia, thereby, effectively amplifying the signal for microglial activation. Ultimately, this effect can lead to recruitment of microglia to the site of injury and can promote a timely resolution of cellular injury, an overly sensitive inflammatory response, or both (Siao and Tsirka 2002). For example, after Ca^{2+}-induced neuronal secretion, tPA cleaves an N-terminal residue on the NR1 subunit of the NMDA receptor, exacerbating NMDA receptor-evoked Ca^{2+} influx during excitotoxic processes (Nicole *et al.* 2001; Fernandez-Monreal *et al.* 2004a), and consequently, propagating an overly sensitive inflammatory response. Alternatively, activated microglia and apoptotic neuronal cells also synthesize PGn, in addition to tPA (Nakajima *et al.* 1992a,b,c; Tsirka *et al.* 1997). In general, tPA secretion alone is not sufficient to cause neuronal degeneration during excitotoxic insult (Tsirka *et al.* 1996, 1997); therefore, presenting PGn on the membrane surface could either provide a way to further localize activated microglia to areas in which neurons have been injured, or suggests that neuronal secretion of tPA is not necessarily intended to be detrimental.

As described previously, PGn has been implicated in inflammation, as well as many intracellular signaling

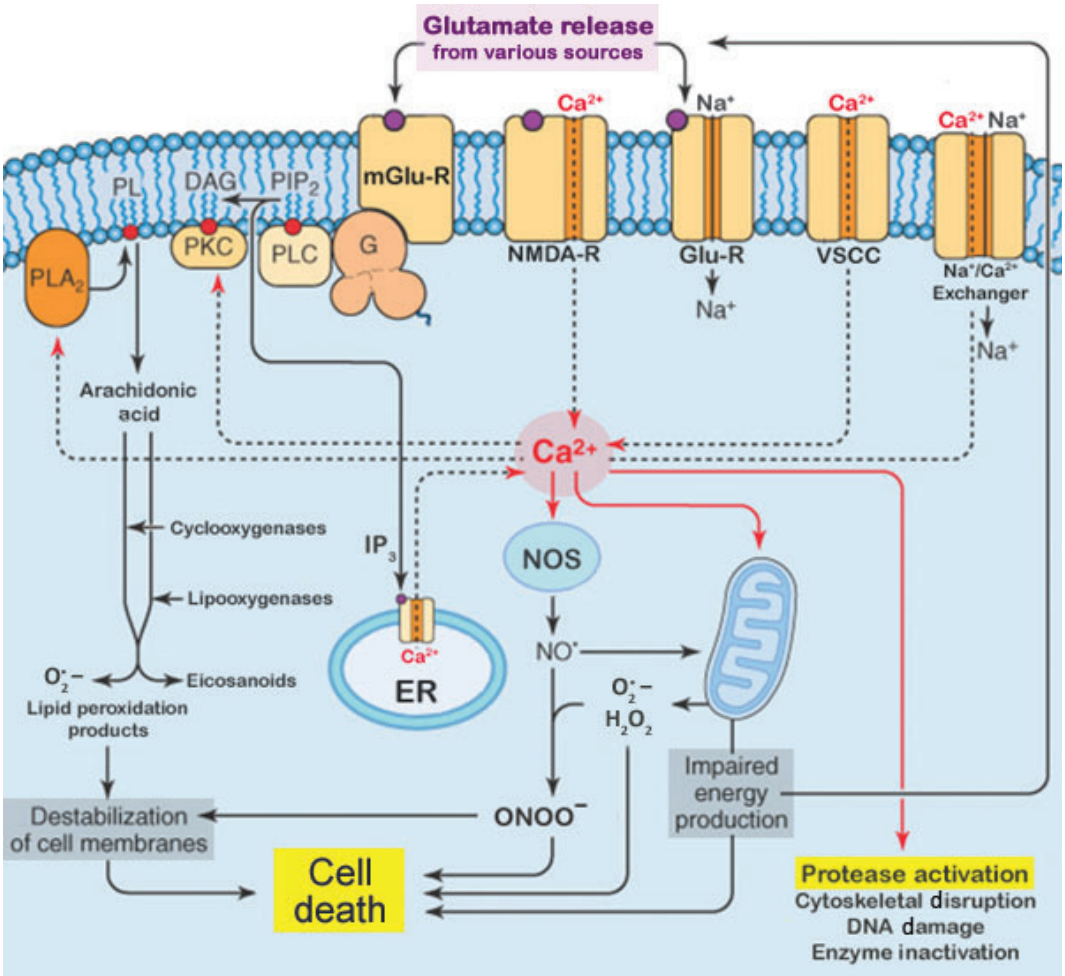

Fig. 3 Glutamate excitotoxicity. This diagram depicts the many intracellular signaling events elicited by excess release and impaired uptake of glutamate, leading to neuronal death. Glu-R, AMPA/kainate receptors; mGlu-R, metabotropic glutamate receptor; NMDA-R, NMDA receptor; VSCC, voltage-sensitive Ca^{2+} channel; PL, phospholipids; DAG, diacylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; G, G-protein; PLA_2, phospholipase A_2; PLC, phospholipase C; PKC, protein kinase C; ER, endoplasmic reticulum; H_2O_2, hydrogen peroxide; $NO^{\bullet}$, nitric oxide; $ONOO^{-}$, peroxynitrite; NOS, nitric oxide synthase; $O_2^{\bullet -}$, superoxide radical [adapted from (Siegel *et al.* 2006)].

events by activation of proenzymes, prohormones, progrowth factors, and procytokines (section Enolase and the plasminogen system). Studies by Nagata *et al.* (1992, 1993) have shown that microglia-derived PGn has a neurotrophic effect on neurons, as purified rat PGn enhanced neurite outgrowth and dopamine uptake of mesencephalic neurons. The effects elicited by PGn were found to be mediated by α-enolase on the neuronal surface (Nagata *et al.* 1993; Nakajima *et al.* 1994). Enolase has frequently been reported as a strong PGn binding protein within the brain (section Enolase and the plasminogen system), because of its extracellular C-terminal Lys residues, and is known to be up-regulated in MCI, EOAD, and AD brain (section Enolase and Alzheimer's disease). When microglial and/or neuronal PGn binds membrane-integrated enolase, PGn is rapidly activated through tPA proteolytic cleavage (section Enolase and the plasminogen system). Consequent production of plasmin endows neurons with the catalytic amplification of tPA/PGn signaling, because of the broad spectrum of substrates affected by proteolytic plasmin (Redlitz *et al.* 1995). Moreover, binding enolase protects plasmin from inactivation from inhibitors, like α_2-antiplasmin (Plow *et al.* 1991; Bergman *et al.* 1997). Therefore, it can be speculated that the up-regulation of enolase, in addition to enhanced membrane-resident PGn and extracellular secretion of tPA, by excitotoxic neurons and/or activated microglia during AD progression may initially be an attempt to propagate neuronal-preservation pathways, that ultimately go awry (Fig. 4).

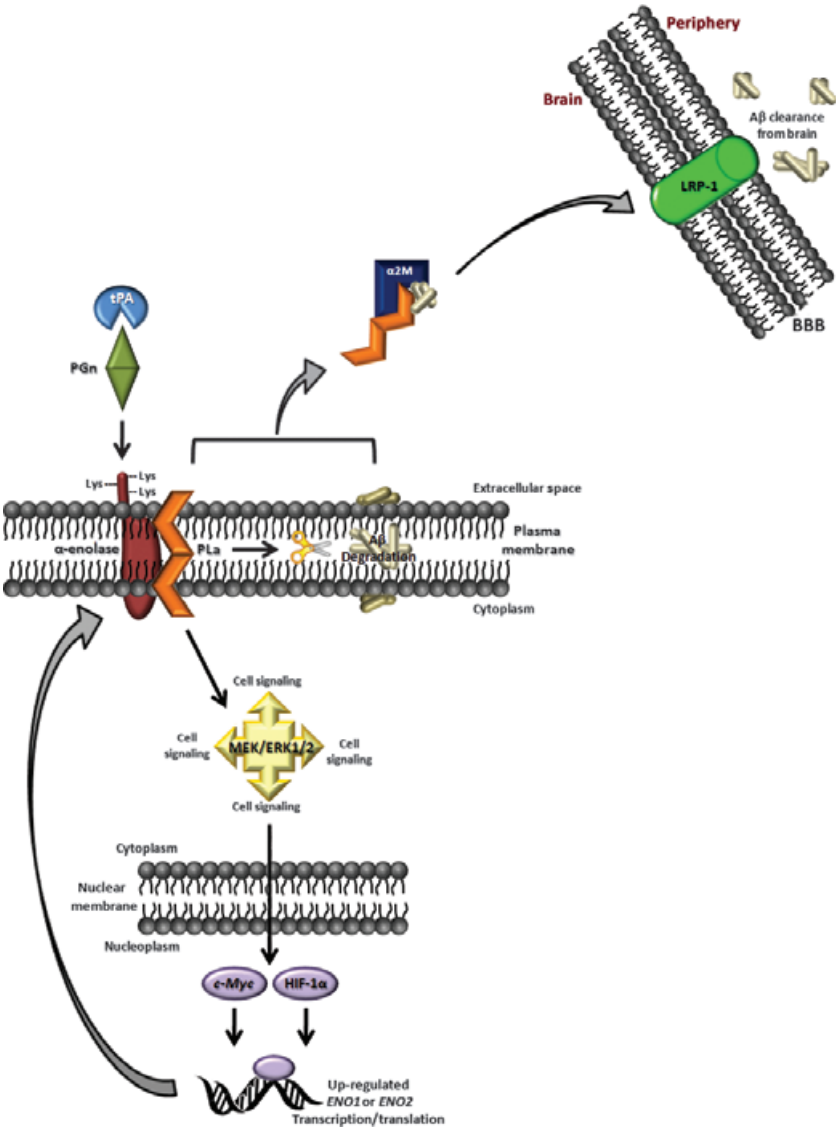

Fig. 4 Possible role of enolase in MCI, EOAD, and AD. This scheme illustrates an alternate role for enolase, in addition to glucose metabolism, in normal and/or MCI, EOAD, and AD brain. In this model, up-regulation and membrane integration of α-enolase, promotes surface-binding of the tPA/PGn complex, which produces the protease plasmin (PLa). Plasmin, in turn, can degrade Aβ peptides associated with the bilayer and activate the MAPK/MEK/ERK1/2 pathway, promoting up-regulation of ENO1 transcription, and therefore, production of α-enolase. In this way, up-regulation of enolase would catalytically amplify an internal signal for cell survival during AD progression. Moreover, by complexing with α2M, plasmin may also be involved with Aβ clearance from the brain via LRP-1 at the blood-brain barrier (BBB). Unfortunately, because of significant oxidative modification, it is hypothesized that enolase becomes unable to facilitate the initiation of these pathways, which would lead to the augmentation of neuronal death in brain of subjects with MCI, EOAD, and AD versus normal aged brain.

Enolase, the plasminogen system, and MAPK/MEK/ERK1/2 signaling

As previously mentioned, a predominant feature in MCI, EOAD, and AD is the manifestation of glucose hypometabolism, which generally causes the up-regulation of glycolytic enzymes, including enolase, to combat the mounting energy deficit (section Enolase and Alzheimer's disease). Thus, the lack of aerobic metabolism in AD brain is indicative of a widespread hypoxic environment. Many studies have found glycolytic ATP to be of utmost importance to the maintenance of endoplasmic reticulum Ca^{2+} stores, by acting as the chief ATP fuel-source for plasma membrane ion pumps, such as the Na^{+}/K^{+}-ATPase and Ca^{2+}-ATPase, rather than ATP produced by mitochondrial oxidative phosphorylation (Kauppinen *et al.* 1988; Xu *et al.* 1995; Silver and Erecinska 1997; Brorson *et al.* 1999; Kahlert and Reiser 2000). However, neither glycolysis nor oxidative phosphorylation alone is capable of sustaining power to these pumps; therefore, a constant ATP supply must be provided by both pathways in order to prevent membrane depolarization (Kauppinen *et al.* 1988). In addition, collapsing cellular ATP reserves and Na^{+} ion gradients can exacerbate glutamate excitotoxicity, lead to rising intracellular Ca^{2+} levels, and activate intracellular signal pathways that either lead to neuronal death or survival (Fig. 3).

Specifically, intracellular pathways involving activation of the mitogen-activated protein kinase (MAPK) cascade are of particular interest on account of one downstream target, enolase. Among many different proteins that MAPKs can activate by phosphorylation, the extracellular signal-regulated kinase 1/2 (ERK1/2) is known to function in cell survival responses by translocating to the nucleus and inducing rapid gene expression (Davis 1993; Karin 1995, 1998; Chang and Karin 2001), often in response to ROS

(Jimenez *et al.* 1997; Chuang *et al.* 2000; Kishida *et al.* 2005; Conde de la Rosa *et al.* 2006; Kulich *et al.* 2007), a well-known initiator and/or consequence of AD pathology (section Enolase and Alzheimer's disease). In a study by Mizukami, *et al.* (Mizukami *et al.* 2004), ERK1/2 was shown to be involved in the maintenance of intracellular ATP through induction of α-enolase expression, resulting in cardiomyocyte survival in ischemic hypoxia and re-oxygenation (Mizukami *et al.* 2004). Sousa *et al.* (2005) provided additional insight into ERK1/2-induced expression of *ENO1* mRNA, revealing that active PGn on the cell surface activates MAPK and ERK1/2 in fibroblasts, through the proteolytic action of plasmin, which leads directly to the transcriptional regulation of *ENO1*. Their data also demonstrate that PGn-regulated *ENO1* expression is not only restricted to fibroblast cells, suggesting that this signaling cascade, involving up-regulation of α-enolase via MAPK and ERK1/2, probably exists in the brain, as well (Sousa *et al.* 2005). Further evidence to support this notion is that all MAPK pathways, including ERK1/2, are known to be activated in AD brain, as ERK1/2 immunoreactivity can be found in tangle-bearing and non-tangle-bearing neurons (Hyman *et al.* 1994), as well as in dystrophic neurites of senile plaques (Trojanowski *et al.* 1993).

Because over 25 proteins have been identified to be downstream targets of ERK1/2 signaling (Lewis *et al.* 2000), the exact intracellular mechanism by which the MAPK/ERK1/2 survival pathway induces *ENO1* expression has yet to be established. However, two possible mechanisms suggested by Mizukami *et al.* (2004) in the heart, involving *c-Myc* and HIF-1α (sections Enolase functional diversity and Enolase and *c-Myc*), may provide insight into α-enolase regulation in the brain. In their study, Mizukami *et al.* (2004) found that ischemic hypoxia and re-oxygenation induced α-enolase expression in cells transfected with *c-Myc* cDNA, suggesting that ERK1/2 may induce α-enolase expression through *c-Myc*. Induction of *c-Myc* mRNA expression is dependent upon Ets transcription factor binding sites located within the *c-Myc* promoter region that are activated by ERK1/2 phosphorylation (Gupta *et al.* 1993; Brunner *et al.* 1994; McCarthy *et al.* 1997; Cheng *et al.* 1999). Furthermore, like MAPK and ERK1/2, strong, active *c-Myc* immunoreactivity has been noted in a subpopulation of reactive astrocytes, dystrophic neurites of senile plaques, and neurons with neurofibrillary degeneration in AD (Ferrer and Blanco 2000), implicating a role for MAPK/ERK1/2 activation of *c-Myc,* that would, in turn, induce expression of α-enolase in AD. As the *ENO1* promoter region contains two *c-Myc* binding motifs within the ChoRE sequence (section Enolase functional diversity), *c-Myc* can directly transactivate production of α-enolase. Interestingly, activation of *c-Myc* by the ERK1/2 pathway selectively up-regulates α-enolase production over MBP-1, as MBP-1 would inhibit *c-Myc* by binding its promoter (section Enolase and *c-Myc*).

Alternatively, the MAPK/ERK1/2 pathway can also induce α-enolase transcription through the hypoxia-inducible factor, HIF-1α, which up-regulates glycolytic gene expression during hypoxia, a common phenomenon in AD pathology (Aaronson *et al.* 1995; Wang *et al.* 1995; Semenza *et al.* 1996) (section Enolase and Alzheimer's disease). Cells adapt to hypoxic conditions by inducing activation of transcription factors such as HIF-1, a key regulator of oxygen homeostasis that accumulates in response to low cellular oxygen levels (Wang and Semenza 1993a,b; Wang *et al.* 1995). Previous studies indicate that embryonic stem cells deficient in HIF-1α expressed decreased levels of mRNAs encoding over ten different glucose transporters and glycolytic enzymes, including α-enolase (Iyer *et al.* 1998), insinuating HIF-1α protective effects are largely attributable to increased metabolic flow. Yang *et al.* (2005) suggest that HIF-1α up-regulation of glucose transporters and glycolytic enzymes during hypoxia may favor glycolytic ATP over ATP produced by oxidative phosphorylation, thereby compensating for diminished ATP supplies resulting from oxygen-deprived mitochondria. This explanation is also consistent with studies that suggest the chief ATP-fuel-source for many cellular functions comes directly from glycolysis, rather than mitochondrial-produced ATP, as mentioned above (Kauppinen *et al.* 1988; Xu *et al.* 1995; Silver and Erecinska 1997; Brorson *et al.* 1999; Kahlert and Reiser 2000).

During MAPK/ERK1 signaling, ERK1 is reported to phosphorylate the C-terminal transactivation domains of HIF-1α in hypoxic HMEC-1 endothelial cells, stimulating HIF-1α transcriptional activity (Richard *et al.* 1999; Minet *et al.* 2000), thus, demonstrating that up-regulation of enolase by HIF-1α can be controlled by MAPK/ERK1/2 signaling (Semenza *et al.* 1996). Moreover, a study by Soucek *et al.* (2003) noted that over-expression of a non-degradeable form of HIF-1α prevents Aβ(1–42)-induced neurotoxicity. Considering all MAPK pathways, including ERK1/2 and HIF-1α, are activated in AD brain, it is possible that up-regulation of enolase through MAPK/ERK1/2 signaling serves a direct neuroprotective function in AD. However, it should be noted that although there are multiple neuroprotective benefits to MAPK/ERK1/2 activation, the *over*-activation of these kinases, especially with respect to cell type, varying apoptotic signals, and diverse downstream targets, can increase sensitivity to neurodegeneration, especially during oxidative insult (Slevin *et al.* 2000; Zhu *et al.* 2002a,b; Chu *et al.* 2004).

Because the ChoRE sequence of many glycolytic genes, including *ENO1*, is analogous to the binding site for *c-Myc* and HIF-1α (section Enolase functional diversity), both of these transcription factors are able to up-regulate glucose metabolism via ERK1/2 signaling under hypoxic/hypometabolic conditions (Semenza *et al.* 1996). For that reason, it is quite possible that both mechanisms are utilized under hypometabolic/hypoxic conditions, either separately

or simultaneously, in MCI, EOAD, and AD brain. Therefore, we speculate that the increased levels of enolase found in MCI, EOAD, and AD brain are attributable to neuronal and/or glial intracellular survival pathways induced by excitotoxic, hypoxic, and/or oxidative stress. Extracellular tPA cleavage of PGn bound to membrane-resident enolase stimulates plasmin activation of the MAPK ERK1/2 pro-survival pathway, that, in turn, up-regulates transcription of glycolytic enzymes, like enolase, in an effort to counteract the hypometabolic imbalance of ATP and critical ion gradients, and perhaps saving the cell from an apoptotic death (Fig. 4). Enhancing the translation of α-enolase, in turn, would allow for additional tPA/PGn binding, thus, perpetuating catalytic amplification of not only MAPK/ERK1/2 survival signaling, but perhaps other self-preservation pathways as well.

Enolase, the plasminogen system, and Aβ

Alzheimer's disease is pathologically characterized by increased levels of oxidative stress and damage, as well as the accumulation of neurofibrillary tangles and amyloid plaques, that ultimately lead to synapse and neuronal cell loss (section Enolase and Alzheimer's disease). Amyloid plaques are the result of an over-accumulation of the amyloid-β (1–40) and/or (1–42) peptides (Aβ), derived from β- and γ-secretase cleavage of the AβPP. As AD pathology progresses, the more toxic Aβ(1–42), in particular, rapidly aggregates into fibrils in a β-sheet conformation, similar to the cross-β-structure that fibrin peptides adopt during fibrinolysis (Kranenburg *et al.* 2002). Interestingly, it is this β-sheet conformation that endows fibrin the ability to bind and activate tPA in the peripheral nervous system (PNS) (Kranenburg *et al.* 2002); yet, thus far, fibrin has not been found in the brain (Dotti *et al.* 2004). Although fibrin and Aβ(1–42) have no relative sequence similarity, Aβ(1–42) is able to bind and activate tPA through its aggregated β-sheet structure, thereby substituting for fibrin in PGn activation by tPA, but not uPA, in the brain (Kingston *et al.* 1995; Wnendt *et al.* 1997).

Studies by Tucker *et al.* (2000a,b), suggest that Aβ accumulation ultimately leads to the activation of the tPA/PGn system by inducing tPA expression *in vitro* and *in vivo*, in a positive feedback-loop manner. Through tPA cleavage of PGn, activated plasmin can degrade oligomeric and fibrillar Aβ, effectively blocking Aβ neuronal toxicity. Van Nostrand and Porter (1999) further demonstrated that plasmin cleavage yields an N-terminal truncated form of Aβ with altered β-sheet properties that enhanced stimulation of tPA activity in a positive feedback-loop manner. Interestingly, plasmin has been noted to preferentially increase α-cleavage of AβPP (forming neurotrophic sAβPPα), either by cleaving AβPP directly, or by activating other proteases (Ledesma *et al.* 2000). Considering that plasmin is known to have an affinity for Lys residues (Weinstein and Doolittle 1972), and can activate metalloproteinases (Kleiner and Stetler-Stevenson 1993), such as candidate α-secretases, ADAM 10 and TACE (Buxbaum *et al.* 1998; Lammich *et al.* 1999), plasmin could contribute to Aβ degradation in two ways. First, plasmin may enhance AβPP α-cleavage, increasing the production the non-toxic sAβPPα over the more toxic Aβ(1–42), and/or, second, by directly degrading all forms of Aβ produced, including sAβPPα, p3, Aβ(1–40), and Aβ (1–42) in the form of oligomers and fibrils (Ledesma *et al.* 2000). In contrast, Melchor *et al.* (Melchor *et al.* 2003) demonstrated a significant decrease in tPA activity in the hippocampus and amygdala of AD patients, implying that diminished plasmin levels are not a consequence of Aβ deposition, but, rather, a cause. These researchers also reported that Aβ accumulation exacerbates diminishing tPA activity by inducing expression of plasminogen activator inhibitor (PAI-1) (Melchor *et al.* 2003), a potent tPA inhibitor (Gils and Declerck 1997).

However, these papers do not discuss the effects of alternatively spliced AβPP derivatives on PGn processing in AD brain, specifically isoforms containing the Kunitz-type serine protease inhibitor (KPI) domains. Alternative splicing of the gene encoding AβPP on chromosome 21 yields three AβPP isoforms of 695 (KPI(−)AβPP), 751, and 770 amino acids; of which, the 751 and 770 amino acid species [KPI(+)AβPP] contain a 56 amino acid KPI domain (Ponte *et al.* 1988; Tanzi *et al.* 1988). KPI domains are highly analogous to the proteinase inhibitor, protease-nexin II and are known to potently inhibit serine proteases, such as prothrombic enzymes and plasmin, but not uPA or tPA (Van Nostrand *et al.* 1989, 1990; Smith *et al.* 1990; Schmaier *et al.* 1993; Shimokawa *et al.* 1993; Mahdi *et al.* 1995; Konduri *et al.* 2001; Xu *et al.* 2005, 2009). In AD subjects, KPI(+)AβPP mRNA and protein levels are significantly elevated in many areas of the brain and CSF, found in senile plaques, and are associated with increased production of Aβ, while KPI(-)AβPP levels are significantly reduced (Palmert *et al.* 1989a,b; Kitaguchi *et al.* 1990; Hyman *et al.* 1992; Saito *et al.* 1993; Willoughby *et al.* 1995; Zhan *et al.* 1995; Moir *et al.* 1998; Preece *et al.* 2004).

Therefore, the above-mentioned models of tPA and plasmin regulation (Van Nostrand *et al.* 1990; Shimokawa *et al.* 1993; Van Nostrand and Porter 1999; Tucker *et al.* 2000a,b; Konduri *et al.* 2001; Melchor *et al.* 2003; Menendez-Gonzalez *et al.* 2005) are not necessarily incompatible, as there might be a negative feedback-loop mechanism between tPA and/or plasmin activity and Aβ deposition. For example, plaque formation may, indeed, trigger the up-regulation of PGn, but the loss of tPA activity (i.e., by PAI-1) prior to AD onset might render a positive feedback-loop (as mentioned above) ineffective (Cacquevel *et al.* 2007). Moreover, increased levels of KPI(+)AβPP in AD brain would serve to exacerbate this negative feedback-loop, wherein both tPA and plasmin activity are inhibited by AD pathology. Thus, oxidative modification of α-enolase and/or other proteins results in the up-regulation of α-enolase during AD progression and may be

an integral part of a system in which neurons attempt to degrade accumulating Aβ. However, the oxidative modification of α-enolase in MCI, EOAD, and AD may render this enzyme either incapable of binding PGn/plasmin while membrane-integrated, or completely unable to integrate into the plasma membrane in order to initiate a PGn/plasmin proteolytic survival cascade. In either case, plasmin would be unable to effectively degrade Aβ or initiate the MAPK/ERK1/2 survival pathway. Consequently, free plasmin proteolytic activity could be inhibited by α_2-antiplasmin (section Enolase and the plasminogen system; Fig. 4).

Lastly, Aβ accumulation in MCI, EOAD, and AD brain can arise from overproduction, decreased degradation (i.e., by plasmin, neprolysin, or other proteases), and/or by a third mechanism: decreased efflux from the brain. Efflux of brain-resident Aβ is primarily facilitated by the low-density lipoprotein-related receptor-1 (LRP-1), which mediates endocytic processing of both secreted and transmembrane forms of AβPP through the blood-brain barrier (Kounnas *et al.* 1995; Knauer *et al.* 1996). Interestingly, studies demonstrate that plasmin interacts with α_2-macroglobulin (α2M), a 'pan-protease inhibitor' (Bu *et al.* 1992; Kovacs 2000) that is bound and internalized by LRP when complexed with proteases, such as plasmin (Rebeck *et al.* 1995; Qiu *et al.* 1996). α2M is an atypical protease inhibitor, in that cleavage of its 'bait region' traps proteases, but does not block or alter the protease active-site or proteolytic ability (Borth 1992; Kovacs 2000). Studies by Qiu *et al.* (1996) demonstrate that a 700 kDa α2M-serine protease complex is responsible for significant Aβ(1–40) and Aβ(1–42) degradation and clearance from the brain. Although, the identity of this particular serine protease is unknown, it is conceivable that plasmin, a serine protease known to bind and degrade Aβ proteins, may be a likely candidate.

Hence, oxidative dysfunction and consequent altered binding of membrane-integrated enolase by PGn in MCI, EOAD, and AD brain could conceivably lead to decreased efflux of brain-resident Aβ. Altered binding of PGn to enolase at the cell surface could significantly reduce or completely inhibit production of the tPA/PGn cleavage product, plasmin, precluding the potential association of α2M with plasmin, and, therefore, Aβ clearance via LRP. Furthermore, as LRP is also a receptor for free and/or α2M-complexed KPI(+)AβPP, which competitively inhibits clearance of Aβ when bound to LRP (Kounnas *et al.* 1995; Ulery *et al.* 2000; Conboy *et al.* 2005; Moir and Tanzi 2005), elevated KPI(+)AβPP levels in AD brain, in addition to altered PGn-enolase binding, may exacerbate decreased efflux of Aβ. Studies to test these notions are now underway in our laboratory.

Enolase in other neurodegenerative diseases

Alzheimer's disease is just one of many age-related neurodegenerative disorders exhibiting a progressive decline in cognitive function, as well as extensive synapse and neuronal cell loss. Shared clinical characteristics between diseases such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and Niemann-Pick disease involve common pathologies including oxidative stress and damage, mitochondrial dysfunction, hindered protein degradation, abnormal intracellular signaling, and cell-cycle arrest (Beal 1995; Jenner and Olanow 1998; Cassarino *et al.* 2000; Duda *et al.* 2000; McNaught and Jenner 2001). Unfortunately, not much research to date has focused on the expression, function, and/or oxidation state of the enzyme enolase in these other neurodegenerative diseases, although many studies have utilized neural-specific enolase (NSE) as a marker for global neuronal and glial cell loss. Studies conducted by our laboratory, among a few others, however, have shown decreased activity, as well as increased expression and oxidative modification of α- and γ-enolase in mouse models of HD (Perluigi *et al.* 2005b; Sorolla *et al.* 2008), PD (De Iuliis *et al.* 2005; Poon *et al.* 2005; Stauber *et al.* 2008; Gomez and Ferrer 2009), DLB (Gomez and Ferrer 2009), and fALS (Casoni *et al.* 2005; Perluigi *et al.* 2005a), in addition to AD (section Enolase and Alzheimer's disease). Conversely, it is interesting to note that α-enolase expression is down-regulated in the striatum of maneb- and paraquat-induced PD mouse models (Patel *et al.* 2007), revealing that the specific route of pathologic cell signaling, oxidative modification, and protein expression may depend upon which factors, genetic and/or environmental, ultimately induce onset of disease pathology.

Considering that hypometabolism/hypoxia, excitotoxicity, the plasminogen system, MAPK/ERK1/2 signaling, HIF-1α, and *c-Myc* have all been implicated as being both protective and deleterious in the pathologies of HD, PD, ALS, DLB, and Niemann-Pick disease (Ferrer and Blanco 2000; Berding *et al.* 2001; Ferrer *et al.* 2001a,b,c; Zhu *et al.* 2002a, 2003; Yang *et al.* 2005; Apostol *et al.* 2006; Demestre *et al.* 2006; Glas *et al.* 2007; Kulich *et al.* 2007; Varma *et al.* 2007; Beal 2008), it is quite likely that enolase dysfunction, dysregulation, and oxidative modification reported by our laboratory in the aforementioned tauopathies and Lewy body variants is a result of similar, if not identical, protective pathways suggested for MCI, EOAD, and AD above. However, more studies are needed to investigate this notion/theory as the plasminogen system, MAPK/ERK1/2, HIF-1α, and *c-Myc* signaling may all act differently in each neurodegenerative disease, in response/accordance to different cell types, apoptotic stimuli, and available downstream targets.

Conclusion

Although the main cause(s) of AD remain unknown, it is evident that up-regulation and loss of activity of glycolytic enzymes, like enolase, is significant to disease progression.

Results from our laboratory support the view that enolase is more than just a glycolytic enzyme, but possesses other functions critical to brain cell survival. In this review we propose an expanded role for enolase that occurs concurrently with up-regulation of this enzyme in AD brain, a role to promote neuronal protection from Aβ accumulation and possibly glutamate excitotoxicity via action of the PGn and MAPK/ERK1/2 systems. However, given that oxidative modification of enzymes generally leads to dysfunction (Butterfield *et al.* 2007), these putative roles of enolase to protect against neuronal death in AD brain fail. Moreover, noting that both harmful and neuroprotective effects of both the MAPK/ERK1/2 and PGn systems in the brain are known, it is evident that the ability of tPA/PGn and plasmin to modulate neuronal death or survival via MAPK/ERK1/2 and enolase in MCI and/or AD would depend upon the cell type and apoptotic stimulus (Tucker *et al.* 2000b). In addition, as our laboratory has identified enolase to be one of the most oxidatively modified proteins in MCI, EOAD, and AD (Castegna *et al.* 2002, 2003; Butterfield *et al.* 2006a,b; Sultana *et al.* 2006a,b; Newman *et al.* 2007; Reed *et al.* 2008, 2009; Perluigi *et al.* 2009; Sultana *et al.* 2009), as well as in response to Aβ(1–42) (Boyd-Kimball *et al.* 2005), it is possible that this enzyme becomes either completely unable to integrate into the cell membrane in order to bind PGn, or the oxidative dysfunction of enolase renders this enzyme incapable of binding to PGn/plasmin while membrane-integrated. These scenarios are consistent with the reported decreased levels of plasmin in AD brain (Ledesma *et al.* 2000). Taken together, whether or not the up-regulation of enolase in AD brain stretches beyond the basic need for metabolic ATP remains unknown, but it is highly likely that oxidative dysfunction of multifunctional enolase extends beyond altered glucose metabolism in ways that contribute to biochemical, pathological, and clinical characteristics of AD. If sustained by ongoing studies, this hypothesis would suggest that enolase is a promising therapeutic target of this devastating dementing disorder.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplemental Information.

Table S1. Summary of enolase isoform chromosomal and tissue localization.

Figure S1. Enolase structure. Ribbon view of the human α-enolase enzyme, subunits C and D.

Figure S2. Enolase active-site. View of yeast α-enolase active-site catalytic residues occupied by an equilibrium mixture of 2-PGA and PEP (ball-and-stick).

Figure S3. Enolase-Mg^{2+}-2-PGA Reaction. Enolases use general acid-base catalysis to cause the reversible interconversion of 2-PGA to PEP by the mechanism shown.

Figure S4. Enolase mobile loop. (a) Ball-and-stick view of the S39AMg^{2+} PhAH complex, with an FoFc electron density map contoured at 3s for PhAH, an alternate substrate to 2-PGA.

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References

Aaronson R. M., Graven K. K., Tucci M., McDonald R. J. and Farber H. W. (1995) Non-neuronal enolase is an endothelial hypoxic stress protein. *J. Biol. Chem.* **270**, 27752–27757.

Aksenov M. Y., Aksenova M. V., Butterfield D. A., Geddes J. W. and Markesbery W. R. (2001) Protein oxidation in the brain in Alzheimer's disease. *Neuroscience* **103**, 373–383.

Andrade-Gordon P. and Strickland S. (1986) Interaction of heparin with plasminogen activators and plasminogen: effects on the activation of plasminogen. *Biochemistry* **25**, 4033–4040.

Apostol B. L., Illes K., Pallos J. *et al.* (2006) Mutant huntingtin alters MAPK signaling pathways in PC12 and striatal cells: ERK1/2 protects against mutant huntingtin-associated toxicity. *Hum. Mol. Genet.* **15**, 273–285.

Bader Lange M. L., Cenini G., Piroddi M., Abdul H. M., Sultana R., Galli F., Memo M. and Butterfield D. A. (2008) Loss of phospholipid asymmetry and elevated brain apoptotic protein levels in subjects with amnestic mild cognitive impairment and Alzheimer disease. *Neurobiol. Dis.* **29**, 456–464.

Baranes D., Lederfein D., Huang Y. Y., Chen M., Bailey C. H. and Kandel E. R. (1998) Tissue plasminogen activator contributes to the late phase of LTP and to synaptic growth in the hippocampal mossy fiber pathway. *Neuron* **21**, 813–825.

Beal M. F. (1995) Aging, energy, and oxidative stress in neurodegenerative diseases. *Ann. Neurol.* **38**, 357–366.

Beal M. F. (2008) The urokinase system of plasminogen activator plays a role in amyotrophic lateral sclerosis (ALS) pathogenesis. *Exp. Neurol.* **211**, 332–333.

Bentley D. L. and Groudine M. (1986) A block to elongation is largely responsible for decreased transcription of *c-myc* in differentiated HL60 cells. *Nature* **321**, 702–706.

Berding G., Odin P., Brooks D. J. *et al.* (2001) Resting regional cerebral glucose metabolism in advanced Parkinson's disease studied in the off and on conditions with [(18)F]FDG-PET. *Mov. Disord.* **16**, 1014–1022.

Bergman A. C., Linder C., Sakaguchi K. *et al.* (1997) Increased expression of α-enolase in c-jun transformed rat fibroblasts without increased activation of plasminogen. *FEBS Lett.* **417**, 17–20.

Blasi F., Vassalli J. D. and Dano K. (1987) Urokinase-type plasminogen activator: proenzyme, receptor, and inhibitors. *J. Cell Biol.* **104**, 801–804.

Borth W. (1992) α2-Macroglobulin, a multifunctional binding protein with targeting characteristics. *FASEB J.* **6**, 3345–3353.

Bottalico L. A., Kendrick N. C., Keller A., Li Y. and Tabas I. (1993) Cholesteryl ester loading of mouse peritoneal macrophages is

associated with changes in the expression or modification of specific cellular proteins, including increase in an α-enolase isoform. *Arterioscler. Thromb.* **13**, 264–275.

Boyd-Kimball D., Castegna A., Sultana R., Poon H. F., Petroze R., Lynn B. C., Klein J. B. and Butterfield D. A. (2005) Proteomic identification of proteins oxidized by Aβ(1-42) in synaptosomes: implications for Alzheimer's disease. *Brain Res.* **1044**, 206–215.

Briggs M. R., Kadonaga J. T., Bell S. P. and Tjian R. (1986) Purification and biochemical characterization of the promoter-specific transcription factor, Sp1. *Science* **234**, 47–52.

Brorson J. R., Schumacker P. T. and Zhang H. (1999) Nitric oxide acutely inhibits neuronal energy production. The Committees on Neurobiology and Cell Physiology. *J. Neurosci.* **19**, 147–158.

Brunner D., Ducker K., Oellers N., Hafen E., Scholz H. and Klambt C. (1994) The ETS domain protein pointed-P2 is a target of MAP kinase in the sevenless signal transduction pathway. *Nature* **370**, 386–389.

Bu G., Williams S., Strickland D. K. and Schwartz A. L. (1992) Low density lipoprotein receptor-related protein/α2-macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. *Proc. Natl Acad. Sci. USA* **89**, 7427–7431.

Bulliard C., Zurbriggen R., Tornare J., Faty M., Dastoor Z. and Dreyer J. L. (1997) Purification of a dichlorophenol-indophenol oxidoreductase from rat and bovine synaptic membranes: tight complex association of a glyceraldehyde-3-phosphate dehydrogenase isoform, TOAD64, enolase-γ and aldolase C. *Biochem. J.* **324** (Pt 2), 555–563.

Butterfield D. A. (2002) Amyloid β-peptide (1-42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review. *Free Radic. Res.* **36**, 1307–1313.

Butterfield D. A. and Lauderback C. M. (2002) Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid β-peptide-associated free radical oxidative stress. *Free Radic. Biol. Med.* **32**, 1050–1060.

Butterfield D. A. and Stadtman E. R. (1997) Protein oxidation processes in aging brain. *Adv. Cell. Aging Gerontol.* **2**, 161–191.

Butterfield D. A. and Sultana R. (2007) Redox proteomics identification of oxidatively modified brain proteins in Alzheimer's disease and mild cognitive impairment: insights into the progression of this dementing disorder. *J. Alzheimers Dis.* **12**, 61–72.

Butterfield D. A., Drake J., Pocernich C. and Castegna A. (2001) Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid β-peptide. *Trends Mol. Med.* **7**, 548–554.

Butterfield D. A., Castegna A., Lauderback C. M. and Drake J. (2002) Evidence that amyloid β-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. *Neurobiol. Aging* **23**, 655–664.

Butterfield D. A., Gnjec A., Poon H. F., Castegna A., Pierce W. M., Klein J. B. and Martins R. N. (2006a) Redox proteomics identification of oxidatively modified brain proteins in inherited Alzheimer's disease: an initial assessment. *J. Alzheimers Dis.* **10**, 391–397.

Butterfield D. A., Poon H. F., St Clair D., Keller J. N., Pierce W. M., Klein J. B. and Markesbery W. R. (2006b) Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. *Neurobiol. Dis.* **22**, 223–232.

Butterfield D. A., Reed T., Newman S. F. and Sultana R. (2007) Roles of amyloid β-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radic. Biol. Med.* **43**, 658–677.

Buxbaum J. D., Liu K. N., Luo Y. *et al.* (1998) Evidence that tumor necrosis factor-α converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem.* **273**, 27765–27767.

Cacquevel M., Launay S., Castel H. *et al.* (2007) Ageing and amyloid-β peptide deposition contribute to an impaired brain tissue plasminogen activator activity by different mechanisms. *Neurobiol. Dis.* **27**, 164–173.

Carmeliet P., Schoonjans L., Kieckens L. *et al.* (1994) Physiological consequences of loss of plasminogen activator gene function in mice. *Nature* **368**, 419–424.

Casoni F., Basso M., Massignan T., Gianazza E., Cheroni C., Salmona M., Bendotti C. and Bonetto V. (2005) Protein nitration in a mouse model of familial amyotrophic lateral sclerosis: possible multifunctional role in the pathogenesis. *J. Biol. Chem.* **280**, 16295–16304.

Cassarino D. S., Halvorsen E. M., Swerdlow R. H., Abramova N. N., Parker W. D. Jr, Sturgill T. W. and Bennett J. P. Jr (2000) Interaction among mitochondria, mitogen-activated protein kinases, and nuclear factor-κB in cellular models of Parkinson's disease. *J. Neurochem.* **74**, 1384–1392.

Castegna A., Aksenov M., Thongboonkerd V., Klein J. B., Pierce W. M., Booze R., Markesbery W. R. and Butterfield D. A. (2002) Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related protein 2, α-enolase and heat shock cognate 71. *J. Neurochem.* **82**, 1524–1532.

Castegna A., Thongboonkerd V., Klein J. B., Lynn B., Markesbery W. R. and Butterfield D. A. (2003) Proteomic identification of nitrated proteins in Alzheimer's disease brain. *J. Neurochem.* **85**, 1394–1401.

Cesarman G. M., Guevara C. A. and Hajjar K. A. (1994) An endothelial cell receptor for plasminogen/tissue plasminogen activator (tPA). II. Annexin II-mediated enhancement of tPA-dependent plasminogen activation. *J. Biol. Chem.* **269**, 21198–21203.

Chang L. and Karin M. (2001) Mammalian MAP kinase signalling cascades. *Nature* **410**, 37–40.

Chaudhary D. and Miller D. M. (1995) The *c-myc* promoter binding protein (MBP-1) and TBP bind simultaneously in the minor groove of the *c-myc* P2 promoter. *Biochemistry* **34**, 3438–3445.

Chen Z. L. and Strickland S. (1997) Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. *Cell* **91**, 917–925.

Cheng M., Wang D. and Roussel M. F. (1999) Expression of *c-myc* in response to colony-stimulating factor-1 requires mitogen-activated protein kinase kinase-1. *J. Biol. Chem.* **274**, 6553–6558.

Chu C. T., Levinthal D. J., Kulich S. M., Chalovich E. M. and DeFranco D. B. (2004) Oxidative neuronal injury. The dark side of ERK1/2. *Eur. J. Biochem.* **271**, 2060–2066.

Chuang S. M., Liou G. Y. and Yang J. L. (2000) Activation of JNK, p38 and ERK mitogen-activated protein kinases by chromium(VI) is mediated through oxidative stress but does not affect cytotoxicity. *Carcinogenesis* **21**, 1491–1500.

Citron M., Oltersdorf T., Haass C., McConlogue L., Hung A. Y., Seubert P., Vigo-Pelfrey C., Lieberburg I. and Selkoe D. J. (1992) Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. *Nature* **360**, 672–674.

Collen D. (1999) The plasminogen (fibrinolytic) system. *Thromb. Haemost.* **82**, 259–270.

Conboy L., Murphy K. J. and Regan C. M. (2005) Amyloid precursor protein expression in the rat hippocampal dentate gyrus modulates during memory consolidation. *J. Neurochem.* **95**, 1677–1688.

Conde de la Rosa L., Schoemaker M. H., Vrenken T. E., Buist-Homan M., Havinga R., Jansen P. L. and Moshage H. (2006) Superoxide anions and hydrogen peroxide induce hepatocyte death by different mechanisms: involvement of JNK and ERK MAP kinases. *J. Hepatol.* **44**, 918–929.

Cooper J. A., Esch F. S., Taylor S. S. and Hunter T. (1984) Phosphorylation sites in enolase and lactate dehydrogenase utilized by tyrosine kinases *in vivo* and *in vitro*. *J. Biol. Chem.* **259**, 7835–7841.

Dang C. V. (1999) *c-Myc* target genes involved in cell growth, apoptosis, and metabolism. *Mol. Cell. Biol.* **19**, 1–11.

Davis R. J. (1993) The mitogen-activated protein kinase signal transduction pathway. *J. Biol. Chem.* **268**, 14553–14556.

De Iuliis A., Grigoletto J., Recchia A., Giusti P. and Arslan P. (2005) A proteomic approach in the study of an animal model of Parkinson's disease. *Clin. Chim. Acta* **357**, 202–209.

De Sousa L. P., Brasil B. S., Silva B. M., Freitas M. H., Nogueira S. V., Ferreira P. C., Kroon E. G. and Bonjardim C. A. (2005) Plasminogen/plasmin regulates c-fos and egr-1 expression via the MEK/ERK pathway. *Biochem. Biophys. Res. Commun.* **329**, 237–245.

Demestre M., Howard R. S., Orrell R. W. and Pullen A. H. (2006) Serine proteases purified from sera of patients with amyotrophic lateral sclerosis (ALS) induce contrasting cytopathology in murine motoneurones to IgG. *Neuropathol. Appl. Neurobiol.* **32**, 141–156.

Dotti C. G., Galvan C. and Ledesma M. D. (2004) Plasmin deficiency in Alzheimer's disease brains: causal or casual? *Neurodegener. Dis.* **1**, 205–212.

Duda J. E., Giasson B. I., Chen Q. *et al.* (2000) Widespread nitration of pathological inclusions in neurodegenerative synucleinopathies. *Am. J. Pathol.* **157**, 1439–1445.

Evan G. I., Wyllie A. H., Gilbert C. S., Littlewood T. D., Land H., Brooks M., Waters C. M., Penn L. Z. and Hancock D. C. (1992) Induction of apoptosis in fibroblasts by *c-myc* protein. *Cell* **69**, 119–128.

Feo S., Arcuri D., Piddini E., Passantino R. and Giallongo A. (2000) *ENO1* gene product binds to the *c-myc* promoter and acts as a transcriptional repressor: relationship with *Myc* promoter-binding protein 1 (MBP-1). *FEBS Lett.* **473**, 47–52.

Fernandez-Monreal M., Lopez-Atalaya J. P., Benchenane K. *et al.* (2004a) Arginine 260 of the amino-terminal domain of NR1 subunit is critical for tissue-type plasminogen activator-mediated enhancement of N-methyl-D-aspartate receptor signaling. *J. Biol. Chem.* **279**, 50850–50856.

Fernandez-Monreal M., Lopez-Atalaya J. P., Benchenane K. *et al.* (2004b) Is tissue-type plasminogen activator a neuromodulator? *Mol. Cell. Neurosci.* **25**, 594–601.

Ferrer I. and Blanco R. (2000) *n-Myc* and *c-myc* expression in Alzheimer disease, Huntington disease and Parkinson disease. *Brain Res. Mol. Brain Res.* **77**, 270–276.

Ferrer I., Blanco R., Carmona M. and Puig B. (2001a) Phosphorylated *c-myc* expression in Alzheimer disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration. *Neuropathol. Appl. Neurobiol.* **27**, 343–351.

Ferrer I., Blanco R., Carmona M., Puig B., Barrachina M., Gomez C. and Ambrosio S. (2001b) Active, phosphorylation-dependent mitogen-activated protein kinase (MAPK/ERK), stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and p38 kinase expression in Parkinson's disease and Dementia with Lewy bodies. *J. Neural Transm.* **108**, 1383–1396.

Ferrer I., Blanco R., Carmona M. *et al.* (2001c) Phosphorylated map kinase (ERK1, ERK2) expression is associated with early tau deposition in neurones and glial cells, but not with increased nuclear DNA vulnerability and cell death, in Alzheimer disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration. *Brain Pathol.* **11**, 144–158.

Fuhrmann G. F., Fehlau R., Schneider H. and Knauf P. A. (1989) The effect of ferricyanide with iodoacetate in calcium-free solution on passive cation permeability in human red blood cells: comparison with the Gardos-effect and with the influence of PCMBS on passive cation permeability. *Biochim. Biophys. Acta* **983**, 179–185.

Giallongo A., Feo S., Moore R., Croce C. M. and Showe L. C. (1986) Molecular cloning and nucleotide sequence of a full-length cDNA for human α-enolase. *Proc. Natl Acad. Sci. USA* **83**, 6741–6745.

Giallongo A., Oliva D., Cali L., Barba G., Barbieri G. and Feo S. (1990) Structure of the human gene for α-enolase. *Eur. J. Biochem.* **190**, 567–573.

al-Giery A. G. and Brewer J. M. (1992) Characterization of the interaction of yeast enolase with polynucleotides. *Biochim. Biophys. Acta* **1159**, 134–140.

Gils A. and Declerck P. J. (1997) Proteinase specificity and functional diversity in point mutants of plasminogen activator inhibitor 1. *J. Biol. Chem.* **272**, 12662–12666.

Glas M., Popp B., Angele B., Koedel U., Chahli C., Schmalix W. A., Anneser J. M., Pfister H. W. and Lorenzl S. (2007) A role for the urokinase-type plasminogen activator system in amyotrophic lateral sclerosis. *Exp. Neurol.* **207**, 350–356.

Gomez A. and Ferrer I. (2009) Increased oxidation of certain glycolysis and energy metabolism enzymes in the frontal cortex in Lewy body diseases. *J. Neurosci. Res.* **87**, 1002–1013.

Grayson D. R., Costa R. H., Xanthopoulos K. G. and Darnell J. E. (1988) One factor recognizes the liver-specific enhancers in α1-antitrypsin and transthyretin genes. *Science* **239**, 786–788.

Gualandris A., Jones T. E., Strickland S. and Tsirka S. E. (1996) Membrane depolarization induces calcium-dependent secretion of tissue plasminogen activator. *J. Neurosci.* **16**, 2220–2225.

Gupta S., Seth A. and Davis R. J. (1993) Transactivation of gene expression by Myc is inhibited by mutation at the phosphorylation sites Thr-58 and Ser-62. *Proc. Natl Acad. Sci. USA* **90**, 3216–3220.

Hajjar K. A., Jacovina A. T. and Chacko J. (1994) An endothelial cell receptor for plasminogen/tissue plasminogen activator. I. Identity with annexin II. *J. Biol. Chem.* **269**, 21191–21197.

Hattori T., Ohsawa K., Mizuno Y., Kato K. and Kohsaka S. (1994) Synthetic peptide corresponding to 30 amino acids of the C-terminal of neuron-specific enolase promotes survival of neocortical neurons in culture. *Biochem. Biophys. Res. Commun.* **202**, 25–30.

Hattori T., Takei N., Mizuno Y., Kato K. and Kohsaka S. (1995) Neurotrophic and neuroprotective effects of neuron-specific enolase on cultured neurons from embryonic rat brain. *Neurosci. Res.* **21**, 191–198.

von Heijne G., Liljestrom P., Mikus P., Andersson H. and Ny T. (1991) The efficiency of the uncleaved secretion signal in the plasminogen activator inhibitor type 2 protein can be enhanced by point mutations that increase its hydrophobicity. *J. Biol. Chem.* **266**, 15240–15243.

Holland J. P., Labieniec L., Swimmer C. and Holland M. J. (1983) Homologous nucleotide sequences at the 5′ termini of messenger RNAs synthesized from the yeast enolase and glyceraldehyde-3-phosphate dehydrogenase gene families. The primary structure of a third yeast glyceraldehyde-3-phosphate dehydrogenase gene. *J. Biol. Chem.* **258**, 5291–5299.

Hurlin P. J. and Dezfouli S. (2004) Functions of Myc:Max in the control of cell proliferation and tumorigenesis. *Int. Rev. Cytol.* **238**, 183–226.

Hyman B. T., Tanzi R. E., Marzloff K., Barbour R. and Schenk D. (1992) Kunitz protease inhibitor-containing amyloid-β protein precursor immunoreactivity in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **51**, 76–83.

Hyman B. T., Elvhage T. E. and Reiter J. (1994) Extracellular signal regulated kinases. Localization of protein and mRNA in the human hippocampal formation in Alzheimer's disease. *Am. J. Pathol.* **144**, 565–572.

Iida H. and Yahara I. (1985) Yeast heat-shock protein of M_r 48,000 is an isoprotein of enolase. *Nature* **315**, 688–690.

Iyer N. V., Kotch L. E., Agani F. *et al.* (1998) Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1α. *Genes Dev.* **12**, 149–162.

Jenner P. and Olanow C. W. (1998) Understanding cell death in Parkinson's disease. *Ann. Neurol.* **44**, S72–S84.

Jimenez L. A., Zanella C., Fung H., Janssen Y. M., Vacek P., Charland C., Goldberg J. and Mossman B. T. (1997) Role of extracellular signal-regulated protein kinases in apoptosis by asbestos and H_2O_2. *Am. J. Physiol.* **273**, L1029–L1035.

Jones N. C., Rigby P. W. and Ziff E. B. (1988) Trans-acting protein factors and the regulation of eukaryotic transcription: lessons from studies on DNA tumor viruses. *Genes Dev.* **2**, 267–281.

Kahlert S. and Reiser G. (2000) Requirement of glycolytic and mitochondrial energy supply for loading of Ca^{2+} stores and InsP(3)-mediated Ca^{2+} signaling in rat hippocampus astrocytes. *J. Neurosci. Res.* **61**, 409–420.

Kalderon N. (1979) Migration of Schwann cells and wrapping of neurites *in vitro*: a function of protease activity (plasmin) in the growth medium. *Proc. Natl Acad. Sci. USA* **76**, 5992–5996.

Kalderon N. (1982) Role of the plasmin-generating system in the developing nervous tissue: I. Proteolysis as a mitogenic signal for the glial cells. *J. Neurosci. Res.* **8**, 509–519.

Kandel E. R. (2001) The molecular biology of memory storage: a dialogue between genes and synapses. *Science* **294**, 1030–1038.

Karin M. (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *J. Biol. Chem.* **270**, 16483–16486.

Karin M. (1998) Mitogen-activated protein kinase cascades as regulators of stress responses. *Ann. NY Acad. Sci.* **851**, 139–146.

Karp G. (2003) Cell and Molecular Biology. Concepts and Experiments. John Wiley & Sons, Inc., New York, NY, USA.

Kauppinen R. A., Enkvist K., Holopainen I. and Akerman K. E. (1988) Glucose deprivation depolarizes plasma membrane of cultured astrocytes and collapses transmembrane potassium and glutamate gradients. *Neuroscience* **26**, 283–289.

Keller J. N., Schmitt F. A., Scheff S. W., Ding Q., Chen Q., Butterfield D. A. and Markesbery W. R. (2005) Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* **64**, 1152–1156.

Kim J. W. and Dang C. V. (2005) Multifaceted roles of glycolytic enzymes. *Trends Biochem. Sci.* **30**, 142–150.

Kingston I. B., Castro M. J. and Anderson S. (1995) *In vitro* stimulation of tissue-type plasminogen activator by Alzheimer amyloid β-peptide analogues. *Nat. Med.* **1**, 138–142.

Kishida K. T., Pao M., Holland S. M. and Klann E. (2005) NADPH oxidase is required for NMDA receptor-dependent activation of ERK in hippocampal area CA1. *J. Neurochem.* **94**, 299–306.

Kitaguchi N., Tokushima Y., Oishi K., Takahashi Y., Shiojiri S., Nakamura S., Tanaka S., Kodaira R. and Ito H. (1990) Determination of amyloid beta protein precursors harboring active form of proteinase inhibitor domains in cerebrospinal fluid of Alzheimer's disease patients by trypsin-antibody sandwich ELISA. *Biochem. Biophys. Res. Commun.* **166**, 1453–1459.

Kleiner D. E. Jr and Stetler-Stevenson W. G. (1993) Structural biochemistry and activation of matrix metalloproteases. *Curr. Opin. Cell Biol.* **5**, 891–897.

Knauer M. F., Orlando R. A. and Glabe C. G. (1996) Cell surface APP751 forms complexes with protease nexin 2 ligands and is internalized via the low density lipoprotein receptor-related protein (LRP). *Brain Res.* **740**, 6–14.

Konakova M., Hucho F. and Schleuning W. D. (1998) Downstream targets of urokinase-type plasminogen-activator-mediated signal transduction. *Eur. J. Biochem.* **253**, 421–429.

Konduri S. D., Rao C. N., Chandrasekar N. *et al.* (2001) A novel function of tissue factor pathway inhibitor-2 (TFPI-2) in human glioma invasion. *Oncogene* **20**, 6938–6945.

Kounnas M. Z., Moir R. D., Rebeck G. W., Bush A. I., Argraves W. S., Tanzi R. E., Hyman B. T. and Strickland D. K. (1995) LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted β-amyloid precursor protein and mediates its degradation. *Cell* **82**, 331–340.

Kovacs D. M. (2000) α2-Macroglobulin in late-onset Alzheimer's disease. *Exp. Gerontol.* **35**, 473–479.

Kozak M. (1999) Initiation of translation in prokaryotes and eukaryotes. *Gene* **234**, 187–208.

Kranenburg O., Bouma B., Kroon-Batenburg L. M., Reijerkerk A., Wu Y. P., Voest E. E. and Gebbink M. F. (2002) Tissue-type plasminogen activator is a multiligand cross-β structure receptor. *Curr. Biol.* **12**, 1833–1839.

Kulich S. M., Horbinski C., Patel M. and Chu C. T. (2007) 6-Hydroxydopamine induces mitochondrial ERK activation. *Free Radic. Biol. Med.* **43**, 372–383.

Lammich S., Kojro E., Postina R., Gilbert S., Pfeiffer R., Jasionowski M., Haass C. and Fahrenholz F. (1999) Constitutive and regulated α-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl Acad. Sci. USA* **96**, 3922–3927.

Ledesma M. D., Da Silva J. S., Crassaerts K., Delacourte A., De Strooper B. and Dotti C. G. (2000) Brain plasmin enhances APP α-cleavage and Aβ degradation and is reduced in Alzheimer's disease brains. *EMBO Rep.* **1**, 530–535.

Lewis T. S., Hunt J. B., Aveline L. D., Jonscher K. R., Louie D. F., Yeh J. M., Nahreini T. S., Resing K. A. and Ahn N. G. (2000) Identification of novel MAP kinase pathway signaling targets by functional proteomics and mass spectrometry. *Mol. Cell* **6**, 1343–1354.

Liotta L. A., Goldfarb R. H., Brundage R., Siegal G. P., Terranova V. and Garbisa S. (1981) Effect of plasminogen activator (urokinase), plasmin, and thrombin on glycoprotein and collagenous components of basement membrane. *Cancer Res.* **41**, 4629–4636.

Lottenberg R., Broder C. C., Boyle M. D., Kain S. J., Schroeder B. L. and Curtiss R. III (1992) Cloning, sequence analysis, and expression in *Escherichia coli* of a *streptococcal* plasmin receptor. *J. Bacteriol.* **174**, 5204–5210.

Lovell M. A. and Markesbery W. R. (2007) Oxidative damage in mild cognitive impairment and early Alzheimer's disease. *J. Neurosci. Res.* **85**, 3036–3040.

Madani R., Hulo S., Toni N., Madani H., Steimer T., Muller D. and Vassalli J. D. (1999) Enhanced hippocampal long-term potentiation and learning by increased neuronal expression of tissue-type plasminogen activator in transgenic mice. *EMBO J.* **18**, 3007–3012.

Mahdi F., Van Nostrand W. E. and Schmaier A. H. (1995) Protease nexin-2/amyloid β-protein precursor inhibits factor Xa in the prothrombinase complex. *J. Biol. Chem.* **270**, 23468–23474.

Marcu K. B., Bossone S. A. and Patel A. J. (1992) Myc function and regulation. *Annu. Rev. Biochem.* **61**, 809–860.

Markesbery W. R. (1999) The role of oxidative stress in Alzheimer disease. *Arch. Neurol.* **56**, 1449–1452.

Markesbery W. R. and Lovell M. A. (2007) Damage to lipids, proteins, DNA, and RNA in mild cognitive impairment. *Arch. Neurol.* **64**, 954–956.

Markesbery W. R., Kryscio R. J., Lovell M. A. and Morrow J. D. (2005) Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. *Ann. Neurol.* **58**, 730–735.

McCarthy S. A., Chen D., Yang B. S. *et al.* (1997) Rapid phosphorylation of Ets-2 accompanies mitogen-activated protein kinase activation and the induction of heparin-binding epidermal growth

factor gene expression by oncogenic Raf-1. *Mol. Cell. Biol.* **17**, 2401–2412.

McNaught K. S. and Jenner P. (2001) Proteasomal function is impaired in substantia nigra in Parkinson's disease. *Neurosci. Lett.* **297**, 191–194.

Melchor J. P., Pawlak R. and Strickland S. (2003) The tissue plasminogen activator-plasminogen proteolytic cascade accelerates amyloid-β (Aβ) degradation and inhibits Aβ-induced neurodegeneration. *J. Neurosci.* **23**, 8867–8871.

Menendez-Gonzalez M., Perez-Pinera P., Martinez-Rivera M., Calatayud M. T. and Blazquez Menes B. (2005) APP processing and the APP-KPI domain involvement in the amyloid cascade. *Neurodegener. Dis.* **2**, 277–283.

Mielke R., Schroder R., Fink G. R., Kessler J., Herholz K. and Heiss W. D. (1996) Regional cerebral glucose metabolism and postmortem pathology in Alzheimer's disease. *Acta Neuropathol.* **91**, 174–179.

Miles L. A., Dahlberg C. M., Levin E. G. and Plow E. F. (1989) Gangliosides interact directly with plasminogen and urokinase and may mediate binding of these fibrinolytic components to cells. *Biochemistry* **28**, 9337–9343.

Miles L. A., Dahlberg C. M., Plescia J., Felez J., Kato K. and Plow E. F. (1991) Role of cell-surface lysines in plasminogen binding to cells: identification of α-enolase as a candidate plasminogen receptor. *Biochemistry* **30**, 1682–1691.

Minet E., Arnould T., Michel G., Roland I., Mottet D., Raes M., Remacle J. and Michiels C. (2000) ERK activation upon hypoxia: involvement in HIF-1 activation. *FEBS Lett.* **468**, 53–58.

Mizukami Y., Iwamatsu A., Aki T. *et al.* (2004) ERK1/2 regulates intracellular ATP levels through α-enolase expression in cardiomyocytes exposed to ischemic hypoxia and reoxygenation. *J. Biol. Chem.* **279**, 50120–50131.

Moir R. D. and Tanzi R. E. (2005) LRP-mediated clearance of Aβ is inhibited by KPI-containing isoforms of APP. *Curr. Alzheimer Res.* **2**, 269–273.

Moir R. D., Lynch T., Bush A. I. *et al.* (1998) Relative increase in Alzheimer's disease of soluble forms of cerebral Aβ amyloid protein precursor containing the Kunitz protease inhibitory domain. *J. Biol. Chem.* **273**, 5013–5019.

Nagase H., Enghild J. J., Suzuki K. and Salvesen G. (1990) Stepwise activation mechanisms of the precursor of matrix metalloproteinase 3 (stromelysin) by proteinases and (4-aminophenyl)mercuric acetate. *Biochemistry* **29**, 5783–5789.

Nagata K., Nakajima K., Takemoto N., Saito H. and Kohsaka S. (1992) Microglia-derived plasminogen enhances neurite outgrowth from explant culture of rat brain. *Int. J. Dev. Neurosci.* **11**, 227–237.

Nagata K., Nakajima K. and Kohsaka S. (1993) Plasminogen promotes the development of rat mesencephalic dopaminergic neurons *in vitro*. *Brain Res. Dev. Brain Res.* **75**, 31–37.

Nakagawa H., Hatakeyama S., Ikesue A. and Miyai H. (1991) Generation of interleukin-8 by plasmin from AVLPR-interleukin-8, the human fibroblast-derived neutrophil chemotactic factor. *FEBS Lett.* **282**, 412–414.

Nakajima K., Takemoto N. and Kohsaka S. (1992a) Retinoic acid enhances the secretion of plasminogen from cultured rat microglia. *FEBS Lett.* **314**, 167–170.

Nakajima K., Tsuzaki N., Nagata K., Takemoto N. and Kohsaka S. (1992b) Production and secretion of plasminogen in cultured rat brain microglia. *FEBS Lett.* **308**, 179–182.

Nakajima K., Tsuzaki N., Shimojo M., Hamanoue M. and Kohsaka S. (1992c) Microglia isolated from rat brain secrete a urokinase-type plasminogen activator. *Brain Res.* **577**, 285–292.

Nakajima K., Hamanoue M., Takemoto N., Hattori T., Kato K. and Kohsaka S. (1994) Plasminogen binds specifically to α-enolase on rat neuronal plasma membrane. *J. Neurochem.* **63**, 2048–2057.

Nelson D. L. and Cox M. M. (2009) Lehninger Principles of Biochemistry. W. H. Freeman & Co., New York, NY.

Newman S. F., Sultana R., Perluigi M., Coccia R., Cai J., Pierce W. M., Klein J. B., Turner D. M. and Butterfield D. A. (2007) An increase in *S*-glutathionylated proteins in the Alzheimer's disease inferior parietal lobule, a proteomics approach. *J. Neurosci. Res.* **85**, 1506–1514.

Nicole O., Docagne F., Ali C., Margaill I., Carmeliet P., MacKenzie E. T., Vivien D. and Buisson A. (2001) The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. *Nat. Med.* **7**, 59–64.

Onyango P., Lubyova B., Gardellin P., Kurzbauer R. and Weith A. (1998) Molecular cloning and expression analysis of five novel genes in chromosome 1p36. *Genomics* **50**, 187–198.

Osthus R. C., Shim H., Kim S. *et al.* (2000) Deregulation of glucose transporter 1 and glycolytic gene expression by *c-myc*. *J. Biol. Chem.* **275**, 21797–21800.

Palmert M. R., Podlisny M. B., Golde T. E. *et al.* (1989a) The beta amyloid protein precursor: mRNAs, membrane-associated forms, and soluble derivatives. *Prog. Clin. Biol. Res.* **317**, 971–984.

Palmert M. R., Podlisny M. B., Witker D. S., Oltersdorf T., Younkin L. H., Selkoe D. J. and Younkin S. G. (1989b) The β-amyloid protein precursor of Alzheimer disease has soluble derivatives found in human brain and cerebrospinal fluid. *Proc. Natl Acad. Sci. USA* **86**, 6338–6342.

Pancholi V. (2001) Multifunctional α-enolase: its role in diseases. *Cell. Mol. Life Sci.* **58**, 902–920.

Pancholi V. and Fischetti V. A. (1998) α-Enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic *Streptococci*. *J. Biol. Chem.* **273**, 14503–14515.

Pang P. T. and Lu B. (2004) Regulation of late-phase LTP and long-term memory in normal and aging hippocampus: role of secreted proteins tPA and BDNF. *Ageing Res. Rev.* **3**, 407–430.

Parnetti L., Palumbo B., Cardinali L., Loreti F., Chionne F., Cecchetti R. and Senin U. (1995) Cerebrospinal fluid neuron-specific enolase in Alzheimer's disease and vascular dementia. *Neurosci. Lett.* **183**, 43–45.

Patel S., Sinha A. and Singh M. P. (2007) Identification of differentially expressed proteins in striatum of maneb-and paraquat-induced Parkinson's disease phenotype in mouse. *Neurotoxicol. Teratol.* **29**, 578–585.

Perluigi M., Fai Poon H., Hensley K., Pierce W. M., Klein J. B., Calabrese V., De Marco C. and Butterfield D. A. (2005a) Proteomic analysis of 4-hydroxy-2-nonenal-modified proteins in G93A-SOD1 transgenic mice–a model of familial amyotrophic lateral sclerosis. *Free Radic. Biol. Med.* **38**, 960–968.

Perluigi M., Poon H. F., Maragos W., Pierce W. M., Klein J. B., Calabrese V., Cini C., De Marco C. and Butterfield D. A. (2005b) Proteomic analysis of protein expression and oxidative modification in r6/2 transgenic mice: a model of Huntington disease. *Mol. Cell Proteomics* **4**, 1849–1861.

Perluigi M., Sultana R., Cenini G., Di Domenico F., Memo M., Pierce W. M., Coccia R. and Butterfield D. A. (2009) Redox proteomics identification of HNE-modified brain proteins in Alzheimer's disease: role of lipid peroxidation in Alzheimer's disease pathogenesis. *Proteomics Clin. Appl.* **3**, 682–693.

Petersen R. C., Smith G. E., Waring S. C., Ivnik R. J., Tangalos E. G. and Kokmen E. (1999) Mild cognitive impairment: clinical characterization and outcome. *Arch. Neurol.* **56**, 303–308.

Peterson C. L. and Calame K. (1989) Proteins binding to site C2 (μE3) in the immunoglobulin heavy-chain enhancer exist in multiple oligomeric forms. *Mol. Cell. Biol.* **9**, 776–786.

Petrak J., Ivanek R., Toman O., Cmejla R., Cmejlova J., Vyoral D., Zivny J. and Vulpe C. D. (2008) Déjà vu in proteomics. A hit

parade of repeatedly identified differentially expressed proteins. *Proteomics* **8**, 1744–1749.

Plow E. F., Felez J. and Miles L. A. (1991) Cellular regulation of fibrinolysis. *Thromb. Haemost.* **66**, 32–36.

Plow E. F., Herren T., Redlitz A., Miles L. A. and Hoover-Plow J. L. (1995) The cell biology of the plasminogen system. *FASEB J.* **9**, 939–945.

Ponte P., Gonzalez-DeWhitt P., Schilling J. *et al.* (1988) A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors. *Nature* **331**, 525–527.

Poon H. F., Frasier M., Shreve N., Calabrese V., Wolozin B. and Butterfield D. A. (2005) Mitochondrial associated metabolic proteins are selectively oxidized in A30P α-synuclein transgenic mice–a model of familial Parkinson's disease. *Neurobiol. Dis.* **18**, 492–498.

Preece P., Virley D. J., Costandi M., Coombes R., Moss S. J., Mudge A. W., Jazin E. and Cairns N. J. (2004) Amyloid precursor protein mRNA levels in Alzheimer's disease brain. *Brain Res. Mol. Brain Res.* **122**, 1–9.

Qian Z., Gilbert M. E., Colicos M. A., Kandel E. R. and Kuhl D. (1993) Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. *Nature* **361**, 453–457.

Qiu W. Q., Borth W., Ye Z., Haass C., Teplow D. B. and Selkoe D. J. (1996) Degradation of amyloid β-protein by a serine protease-α2-macroglobulin complex. *J. Biol. Chem.* **271**, 8443–8451.

Ray R. B. (1995) Induction of cell death in murine fibroblasts by a *c-myc* promoter binding protein. *Cell Growth Differ.* **6**, 1089–1096.

Ray R. and Miller D. M. (1991) Cloning and characterization of a human c-myc promoter-binding protein. *Mol. Cell. Biol.* **11**, 2154–2161.

Rebeck G. W., Harr S. D., Strickland D. K. and Hyman B. T. (1995) Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein E receptor, the α2-macroglobulin receptor/low-density-lipoprotein receptor-related protein. *Ann. Neurol.* **37**, 211–217.

Redlitz A., Fowler B. J., Plow E. F. and Miles L. A. (1995) The role of an enolase-related molecule in plasminogen binding to cells. *Eur. J. Biochem.* **227**, 407–415.

Reed T., Perluigi M., Sultana R., Pierce W. M., Klein J. B., Turner D. M., Coccia R., Markesbery W. R. and Butterfield D. A. (2008) Redox proteomic identification of 4-hydroxy-2-nonenal-modified brain proteins in amnestic mild cognitive impairment: insight into the role of lipid peroxidation in the progression and pathogenesis of Alzheimer's disease. *Neurobiol. Dis.* **30**, 107–120.

Reed T. T., Pierce W. M. Jr, Turner D. M., Markesbery W. R. and Butterfield D. A. (2009) Proteomic identification of nitrated brain proteins in early Alzheimer's disease inferior parietal lobule. *J. Cell Mol. Med.* (in press).

Richard D. E., Berra E., Gothie E., Roux D. and Pouyssegur J. (1999) p42/p44 Mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1α (HIF-1α) and enhance the transcriptional activity of HIF-1. *J. Biol. Chem.* **274**, 32631–32637.

Rifkin D. B., Moscatelli D., Bizik J., Quarto N., Blei F., Dennis P., Flaumenhaft R. and Mignatti P. (1990) Growth factor control of extracellular proteolysis. *Cell. Differ. Dev.* **32**, 313–318.

Rogove A. D. and Tsirka S. E. (1998) Neurotoxic responses by microglia elicited by excitotoxic injury in the mouse hippocampus. *Curr. Biol.* **8**, 19–25.

Saito F., Yanagisawa K. and Miyatake T. (1993) Soluble derivatives of β/A4 amyloid protein precursor in human cerebrospinal fluid are both N- and O-glycosylated. *Brain Res. Mol. Brain Res.* **19**, 171–174.

Sappino A. P., Madani R., Huarte J., Belin D., Kiss J. Z., Wohlwend A. and Vassalli J. D. (1993) Extracellular proteolysis in the adult murine brain. *J. Clin. Invest.* **92**, 679–685.

Scheuner D., Eckman C., Jensen M. *et al.* (1996) Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the *presenilin-1* and *-2* and *APP* mutations linked to familial Alzheimer's disease. *Nat. Med.* **2**, 864–870.

Schmaier A. H., Dahl L. D., Rozemuller A. J., Roos R. A., Wagner S. L., Chung R. and Van Nostrand W. E. (1993) Protease nexin-2/amyloid-β protein precursor. A tight-binding inhibitor of coagulation factor IXa. *J. Clin. Invest.* **92**, 2540–2545.

Sedoris K. C., Thomas S. D. and Miller D. M. (2007) *c-Myc* promoter binding protein regulates the cellular response to an altered glucose concentration. *Biochemistry* **46**, 8659–8668.

Semenza G. L., Jiang B. H., Leung S. W., Passantino R., Concordet J. P., Maire P. and Giallongo A. (1996) Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J. Biol. Chem.* **271**, 32529–32537.

Shimokawa M., Nakamura K., Maruyama K., Tagawa K., Miyatake T., Sugita H., Ishiura S. and Suzuki K. (1993) Inhibitory spectra of purified protease nexin-II and related proteins towards cellular proteinases. *Biochimie* **75**, 911–915.

Siao C. J. and Tsirka S. E. (2002) Tissue plasminogen activator mediates microglial activation via its finger domain through annexin II. *J. Neurosci.* **22**, 3352–3358.

Siegel G. J., Albers R. W., Brady S. T. and Price D. L. (2006) Basic Neurochemistry. Molecular, Cellular and Medical Aspects. Elsivier Academic Press, Burlington, MA, USA.

Silver I. A. and Erecinska M. (1997) Energetic demands of the Na^+/K^+-ATPase in mammalian astrocytes. *Glia* **21**, 35–45.

Slevin M., Krupinski J., Slowik A., Rubio F., Szczudlik A. and Gaffney J. (2000) Activation of MAP kinase (ERK-1/ERK-2), tyrosine kinase and VEGF in the human brain following acute ischaemic stroke. *Neuroreport* **11**, 2759–2764.

Smith R. P., Higuchi D. A. and Broze G. J. Jr (1990) Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. *Science* **248**, 1126–1128.

Sorolla M. A., Reverter-Branchat G., Tamarit J., Ferrer I., Ros J. and Cabiscol E. (2008) Proteomic and oxidative stress analysis in human brain samples of Huntington disease. *Free Radic. Biol. Med.* **45**, 667–678.

Soucek T., Cumming R., Dargusch R., Maher P. and Schubert D. (2003) The regulation of glucose metabolism by HIF-1 mediates a neuroprotective response to amyloid-β peptide. *Neuron* **39**, 43–56.

Sousa L. P., Silva B. M., Brasil B. S., Nogueira S. V., Ferreira P. C., Kroon E. G., Kato K. and Bonjardim C. A. (2005) Plasminogen/plasmin regulates α-enolase expression through the MEK/ERK pathway. *Biochem. Biophys. Res. Commun.* **337**, 1065–1071.

Spencer C. A. and Groudine M. (1991) Control of *c-myc* regulation in normal and neoplastic cells. *Adv. Cancer Res.* **56**, 1–48.

Stauber J., Lemaire R., Franck J., Bonnel D., Croix D., Day R., Wisztorski M., Fournier I. and Salzet M. (2008) MALDI imaging of formalin-fixed paraffin-embedded tissues: application to model animals of Parkinson disease for biomarker hunting. *J. Proteome Res.* **7**, 969–978.

Sturchler-Pierrat C., Abramowski D., Duke M. *et al.* (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc. Natl Acad. Sci. USA* **94**, 13287–13292.

Subramanian A. and Miller D. M. (2000) Structural analysis of α-enolase. Mapping the functional domains involved in down-regulation of the c-myc protooncogene. *J. Biol. Chem.* **275**, 5958–5965.

Sultana R., Boyd-Kimball D., Poon H. F., Cai J., Pierce W. M., Klein J. B., Merchant M., Markesbery W. R. and Butterfield D. A. (2006a) Redox proteomics identification of oxidized proteins in

Alzheimer's disease hippocampus and cerebellum: an approach to understand pathological and biochemical alterations in AD. *Neurobiol. Aging* **27**, 1564–1576.

Sultana R., Poon H. F., Cai J., Pierce W. M., Merchant M., Klein J. B., Markesbery W. R. and Butterfield D. A. (2006b) Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. *Neurobiol. Dis.* **22**, 76–87.

Sultana R., Boyd-Kimball D., Cai J., Pierce W. M., Klein J. B., Merchant M. and Butterfield D. A. (2007) Proteomics analysis of the Alzheimer's disease hippocampal proteome. *J. Alzheimers Dis.* **11**, 153–164.

Sultana R., Perluigi M., Newman S. F., Pierce W., Cini C., Coccia R. and Butterfield D. A. (2009) Redox proteomic analysis of carbonylated brain proteins in mild cognitive impairment and early Alzheimer's disease. *Antioxidant Redox. Signal. in press.*

Takei N., Kondo J., Nagaike K., Ohsawa K., Kato K. and Kohsaka S. (1991) Neuronal survival factor from bovine brain is identical to neuron-specific enolase. *J. Neurochem.* **57**, 1178–1184.

Tanzi R. E., McClatchey A. I., Lamperti E. D., Villa-Komaroff L., Gusella J. F. and Neve R. L. (1988) Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. *Nature* **331**, 528–530.

Tarui T., Majumdar M., Miles L. A., Ruf W. and Takada Y. (2002) Plasmin-induced migration of endothelial cells. A potential target for the anti-angiogenic action of angiostatin. *J. Biol. Chem.* **277**, 33564–33570.

Thompson K. S. and Towle H. C. (1991) Localization of the carbohydrate response element of the rat L-type pyruvate kinase gene. *J. Biol. Chem.* **266**, 8679–8682.

Toole-Simms W., Sun I. L., Faulk W. P., Low H., Lindgren A., Crane F. L. and Morre D. J. (1991) Inhibition of transplasma membrane electron transport by monoclonal antibodies to the transferrin receptor. *Biochem. Biophys. Res. Commun.* **176**, 1437–1442.

Towle H. C. (1995) Metabolic regulation of gene transcription in mammals. *J. Biol. Chem.* **270**, 23235–23238.

Trojanowski J. Q., Mawal-Dewan M., Schmidt M. L., Martin J. and Lee V. M. (1993) Localization of the mitogen activated protein kinase ERK2 in Alzheimer's disease neurofibrillary tangles and senile plaque neurites. *Brain Res.* **618**, 333–337.

Tsirka S. E., Gualandris A., Amaral D. G. and Strickland S. (1995) Excitotoxin-induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. *Nature* **377**, 340–344.

Tsirka S. E., Rogove A. D. and Strickland S. (1996) Neuronal cell death and tPA. *Nature* **384**, 123–124.

Tsirka S. E., Rogove A. D., Bugge T. H., Degen J. L. and Strickland S. (1997) An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. *J. Neurosci.* **17**, 543–552.

Tucker H. M., Kihiko-Ehmann M., Wright S., Rydel R. E. and Estus S. (2000a) Tissue plasminogen activator requires plasminogen to modulate amyloid-β neurotoxicity and deposition. *J. Neurochem.* **75**, 2172–2177.

Tucker H. M., Kihiko M., Caldwell J. N. *et al.* (2000b) The plasmin system is induced by and degrades amyloid-β aggregates. *J. Neurosci.* **20**, 3937–3946.

Ulery P. G., Beers J., Mikhailenko I., Tanzi R. E., Rebeck G. W., Hyman B. T. and Strickland D. K. (2000) Modulation of β-amyloid precursor protein processing by the low density lipoprotein receptor-related protein (LRP). Evidence that LRP contributes to the pathogenesis of Alzheimer's disease. *J. Biol. Chem.* **275**, 7410–7415.

Van Nostrand W. E. and Porter M. (1999) Plasmin cleavage of the amyloid β-protein: alteration of secondary structure and stimulation of tissue plasminogen activator activity. *Biochemistry* **38**, 11570–11576.

Van Nostrand W. E., Wagner S. L., Suzuki M., Choi B. H., Farrow J. S., Geddes J. W., Cotman C. W. and Cunningham D. D. (1989) Protease nexin-II, a potent antichymotrypsin, shows identity to amyloid β-protein precursor. *Nature* **341**, 546–549.

Van Nostrand W. E., Wagner S. L., Farrow J. S. and Cunningham D. D. (1990) Immunopurification and protease inhibitory properties of protease nexin-2/amyloid β-protein precursor. *J. Biol. Chem.* **265**, 9591–9594.

Varma H., Cheng R., Voisine C., Hart A. C. and Stockwell B. R. (2007) Inhibitors of metabolism rescue cell death in Huntington's disease models. *Proc. Natl Acad. Sci. USA* **104**, 14525–14530.

Virji M. A., Vassalli J. D., Estensen R. D. and Reich E. (1980) Plasminogen activator of islets of Langerhans: modulation by glucose and correlation with insulin production. *Proc. Natl Acad. Sci. USA* **77**, 875–879.

Wang G. L. and Semenza G. L. (1993a) Characterization of hypoxia-inducible factor 1 and regulation of DNA binding activity by hypoxia. *J. Biol. Chem.* **268**, 21513–21518.

Wang G. L. and Semenza G. L. (1993b) General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc. Natl Acad. Sci. USA* **90**, 4304–4308.

Wang G. L., Jiang B. H., Rue E. A. and Semenza G. L. (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc. Natl Acad. Sci. USA* **92**, 5510–5514.

Weinstein M. J. and Doolittle R. F. (1972) Differential specificities of the thrombin, plasmin and trypsin with regard to synthetic and natural substrates and inhibitors. *Biochim. Biophys. Acta* **258**, 577–590.

Willoughby D. A., Rozovsky I., Lo A. C. and Finch C. E. (1995) β-Amyloid precursor protein (APP) and APP-RNA are rapidly affected by glutamate in cultured neurons: selective increase of mRNAs encoding a Kunitz protease inhibitor domain. *J. Mol. Neurosci.* **6**, 257–276.

Winblad B., Palmer K., Kivipelto M. *et al.* (2004) Mild cognitive impairment–beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J. Intern. Med.* **256**, 240–246.

Wisniewski T., Dowjat W. K., Buxbaum J. D. *et al.* (1998) A novel Polish *presenilin-1* mutation (P117L) is associated with familial Alzheimer's disease and leads to death as early as the age of 28 years. *Neuroreport* **9**, 217–221.

Wistow G. J., Lietman T., Williams L. A., Stapel S. O., de Jong W. W., Horwitz J. and Piatigorsky J. (1988) τ-Crystallin/α-enolase: one gene encodes both an enzyme and a lens structural protein. *J. Cell Biol.* **107**, 2729–2736.

Wnendt S., Wetzels I. and Gunzler W. A. (1997) Amyloid β peptides stimulate tissue-type plasminogen activator but not recombinant prourokinase. *Thromb. Res.* **85**, 217–224.

Xu K. Y., Zweier J. L. and Becker L. C. (1995) Functional coupling between glycolysis and sarcoplasmic reticulum Ca^{2+} transport. *Circ. Res.* **77**, 88–97.

Xu F., Davis J., Miao J., Previti M. L., Romanov G., Ziegler K. and Van Nostrand W. E. (2005) Protease nexin-2/amyloid β-protein precursor limits cerebral thrombosis. *Proc. Natl Acad. Sci. USA* **102**, 18135–18140.

Xu F., Previti M. L., Nieman M. T., Davis J., Schmaier A. H. and Van Nostrand W. E. (2009) AβPP/APLP2 family of Kunitz serine proteinase inhibitors regulate cerebral thrombosis. *J. Neurosci.* **29**, 5666–5670.

Yang Y. T., Ju T. C. and Yang D. I. (2005) Induction of hypoxia inducible factor-1 attenuates metabolic insults induced by 3-nitropropionic acid in rat C6 glioma cells. *J. Neurochem.* **93**, 513–525.

Zhan S. S., Sandbrink R., Beyreuther K. and Schmitt H. P. (1995) APP with Kunitz type protease inhibitor domain (KPI) correlates with neuritic plaque density but not with cortical synaptophysin immunoreactivity in Alzheimer's disease and non-demented aged subjects: a multifactorial analysis. *Clin. Neuropathol.* **14**, 142–149.

Zhu J. H., Kulich S. M., Oury T. D. and Chu C. T. (2002a) Cytoplasmic aggregates of phosphorylated extracellular signal-regulated protein kinases in Lewy body diseases. *Am. J. Pathol.* **161**, 2087–2098.

Zhu X., Lee H. G., Raina A. K., Perry G. and Smith M. A. (2002b) The role of mitogen-activated protein kinase pathways in Alzheimer's disease. *Neurosignals* **11**, 270–281.

Zhu J. H., Guo F., Shelburne J., Watkins S. and Chu C. T. (2003) Localization of phosphorylated ERK/MAP kinases to mitochondria and autophagosomes in Lewy body diseases. *Brain Pathol.* **13**, 473–481.

