Mechanisms of Disease: astrocytes in neurodegenerative disease

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SUMMARY

The term neurodegenerative disease refers to the principal pathology associated with disorders such as amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease and Parkinson's disease, and it is presumed that neurodegeneration results in the clinical findings seen in patients with these diseases. Decades of pathological and physiological studies have focused on neuronal abnormalities in these disorders, but it is becoming increasingly evident that astrocytes are also important players in these and other neurological disorders. Our understanding of the normative biology of astrocytes has been aided by the development of animal models in which astrocyte-specific proteins and pathways have been manipulated, and mouse models of neurodegenerative diseases have also revealed astrocyte-specific pathologies that contribute to neurodegeneration. These models have led to the development of targeted therapies for pathways in which astrocytes participate, and this research should ultimately influence the clinical treatment of neurodegenerative disorders.

KEYWORDS astrocytes, glia, glial fibrillary acidic protein, glutamate, neurodegenerative disease

REVIEW CRITERIA

PubMed was searched using Entrez for articles published up to 30 May 2006, including electronic early release publications. Search terms included "astrocyte and neurodegeneration" and "astrocytosis and neurodegeneration". Phrases combining "astrocytes" with individual neurodegenerative disorders, for example, "astrocyte and Alzheimer's disease", were also included in the search. The abstracts of retrieved citations were reviewed and prioritized by relevant content. Full articles were obtained and references were checked for additional material where appropriate.

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INTRODUCTION

Until relatively recently, astrocytes, along with other cells of the glial lineage such as oligodendrocytes and microglia, were believed to be structural cells, the main function of which was to hold neurons together. It is now known, however, that astrocytes serve many housekeeping functions, including maintenance of the extracellular environment and stabilization of cell–cell communications in the CNS. The function of astrocytes in regulating cerebral blood flow and maintaining synaptic function is becoming increasingly recognized as being of paramount importance in the maintenance of the neuronal environment. Astrocytes are also central to the maintenance of neuronal metabolism and neurotransmitter synthesis. Understanding these functions has allowed a refocusing with regard to the role of astrocytes in neurodegenerative diseases, which has led to astrocyte-specific analyses with potential for drug discovery.

CELLULAR FUNCTIONS OF ASTROCYTES

We will begin by highlighting a subset of the many cellular functions of astrocytes, focusing specifically on those functions that have the most relevance to neurodegeneration (Figure 1). Other astrocytic functions, which are beyond the scope of this Review, include the regulation of cell volume, structural support, and the release of neurotransmitters other than glutamate.

Amino acid, nutrient and ion metabolism in the brain

Astrocytes are central to the catabolism of selected amino acids in the brain, as well as to the synthesis of new amino acids. The production of longer carbon backbones in the brain can only occur in astrocytes, owing to the selective localization of pyruvate carboxylase, the only brain enzyme capable of replenishing molecular intermediates for other metabolic reactions.

Astrocytes transport various nutrient and metabolic precursors to neurons via the malate–aspartate shuttle. One of the most important

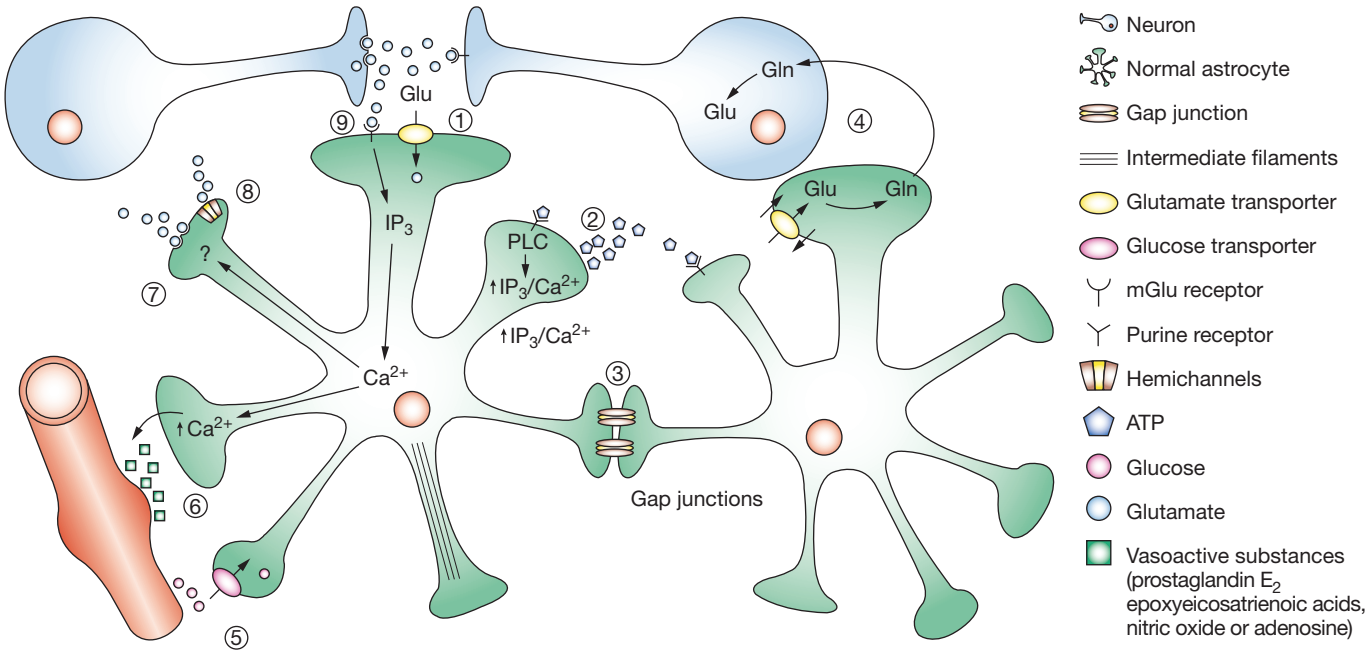

Figure 1 Normal functions of astrocytes. (1) Astrocyte functions include modulation of synaptic function via glutamate transporters, which convey glutamate from the synaptic cleft into the cell.[1] (2) Communication between astrocytes occurs via ATP release and binding to purine receptors on adjacent astrocytes.[14] ATP binding results in phospholipase C activation, with subsequent downstream activation of inositol trisphosphate, resulting in calcium mobilization. (3) Gap junctions contribute to an astrocyte syncytium for the exchange of small molecules and cell–cell communication.[14] Metabolic functions include (4) the replenishment of neuronal glutamate via the glutamate–glutamine cycle, and (5) the transport of glucose from the vasculature.[1] (6) The regulation of blood flow is modulated by astrocyte end-feet apposing blood vessels, with vasodilation being mediated through release of vasoactive substances.[3,4] (7) Glutamate release might occur following elevations in intracellular calcium and the activation of other factors related to prostaglandins.[12] (8) Glutamate release through hemichannels can be induced *in vitro* through lowering of extracellular calcium.[13] (9) Glutamate binding to metabotropic glutamate receptors activates intracellular calcium, leading to the release of vasodilatory substances.[4] Abbreviations: Gln, glutamine; Glu, glutamate; IP_3, inositol trisphosphate; PLC, phospholipase C.

metabolic links between neurons and astrocytes, however, is the glutamate–glutamine shuttle. Astrocytes transport the vast majority of extracellular glutamate (especially neurotransmitter pools) and convert it to glutamine. This glutamine is shuttled back to presynaptic terminals, and is critical for the synthesis of the neurotransmitter glutamate. Astrocytes also convert glucose to lactic acid, which is subsequently taken up into neurons and converted to pyruvate for energy metabolism.[1]

Astrocytes have an important role in the regulation of ion concentrations in the intracellular and extracellular spaces in the brain. Carbon dioxide is produced by neurons following the oxidative metabolism of pyruvate. Astrocytes regulate acid–base balance via carbonic anhydrase, which converts carbon dioxide and water to hydrogen ions and bicarbonate ions. Extracellular potassium also accumulates from neural activity,[2] and buffering of potassium occurs through potassium channels expressed by astrocytes at synapses and at end-foot processes around capillaries.

Coupling of neuronal activity and cerebral blood flow

Evidence is accumulating that astrocytes have an important function in cerebrovascular regulation. Astrocytic processes have end-feet with contact to the brain vasculature, and they envelop neuronal synapses. The relationship between neurons, astrocytes and blood vessels makes astrocytes a central element that can modulate neuronal activity and cerebral blood flow. *In vitro* and *in vivo* studies of cortical tissue indicate that synaptic release of glutamate activates metabotropic glutamate receptors on astrocytes. These receptors trigger the release of arachidonic acid metabolites,[3] leading to a localized increase in calcium at astrocyte end-feet, which results in dilation of nearby arterioles.[4]

Modulation of excitatory synaptic transmission

Glutamate is the primary excitatory neurotransmitter in the CNS, and its activity is carefully regulated by both neuronal and glial influences. The majority of synaptic and perisynaptic glutamate regulation occurs through glutamate transporters. In addition to the tightly coupled synaptic relationship between neuronal synapses and astrocytes, astrocyte-to-astrocyte transmission through gap junctions, as well as paracrine release of ATP, might modulate synaptic biology.

Regulation of glutamate transport

Glutamate transport is a sodium- and potassium-coupled process that is capable of concentrating intracellular glutamate more than 10,000-fold compared with the extracellular environment. The glutamate transporters GLAST and GLT1 (EAAT1 and EAAT2 in humans; also known as EAA1 and EAA2) are localized primarily on astrocyte membranes.[5–8] Antisense knockdown studies showed that these two glial transporters are responsible for over 80% of glutamate uptake in the brain,[9] an observation that was later confirmed in *GLT1* (*Slc1a2*)-null mice.[10]

Release of glutamate

Although glutamate is the primary neuronal excitatory neurotransmitter in the brain, evidence also exists for an astrocytic role in glutamate release. *In vitro* preparations have demonstrated astrocyte-specific glutamate release via exocytosis.[11,12] Certain conditions, such as low levels of extracellular calcium, can trigger glutamate release through a separate mechanism, namely hemichannels—a single cell's contribution to a gap junction.[13] These findings are intriguing in that they suggest an additional layer of fine-tuning of the perisynaptic environment modulated by astrocytes.

Propagation of glutamatergic transmission via the astrocyte network

Current evidence indicates that propagation of glutamate transmission through the astrocyte syncytium occurs through two prominent calcium-mediated mechanisms: one involving gap junctions and the other involving paracrine release of ATP. Activation of metabotropic glutamate receptors on astrocytes following neuronal release of glutamate results in the activation of an inositol trisphosphate (IP_3) pathway, which induces calcium release from intracellular stores. This calcium can then be transferred to the adjacent astrocyte through connexin 43 (Cx43) gap junctions, thereby producing a calcium wave through an astrocyte syncytium. IP_3 also activates ATP release through Cx43 hemichannels. This ATP release acts in a paracrine fashion, activating purine receptors on adjacent astrocytes. This activation results in IP_3 production, more ATP release and intracellular calcium mobilization through a feed-forward mechanism.[14]

ASTROCYTE-SPECIFIC PATHOLOGY: LESSONS FROM MOUSE MODELS

Transgenic and knockout mice have been valuable in helping us to understand the pathophysiology of neurodegenerative disorders. Transgenic models for specific neurodegenerative disorders including amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Huntington's disease (HD) and Parkinson's disease (PD) have been developed, and these models are reviewed later in this article. Some examples of mouse models in which astrocyte protein function is altered are detailed in Table 1.

Manipulations of the astrocytic structural protein glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein that is known to be localized to astrocytes, although its precise contributions to astroglial physiology and function are not clear. The upregulation of GFAP following injury and astrogliosis has been a long-standing pathological observation. Deletion of GFAP in mice did not result in any specific pathology in the absence of injury, but some modest abnormalities were observed following specific injury paradigms.[15] Interestingly, the overexpression of human wild-type GFAP in mice produced pathology consistent with that observed in Alexander disease.[16] The deletion of both GFAP and vimentin, another intermediate filament protein, resulted in more-marked pathology following injury, including a greater loss of neuronal synapses, although it also led to improved regenerative potential.[17] These findings suggest that the significance of GFAP is magnified following injury in which cellular stability might be affected, but that other intermediate filaments have overlapping functions with GFAP.

Disruption of astroglial synaptic regulation: glutamate transporter knockout mice

One of the pivotal functions of astrocytes is the maintenance of glutamate homeostasis

Table 1 Mouse models with altered astrocyte protein function.

Animal model	Mutation or pathway	Pathology	Phenotype
GFAP null	Intermediate filament (GFAP)	Absent intermediate filaments in nonreactive astrocytes Modest abnormalities in some types of injury	Normal behavior and lifespan
GFAP/Vim null	Intermediate filament (GFAP and vimentin)	Absent intermediate filaments in nonreactive astrocytes	Loss of neuronal synapses acutely following injury with increased capacity for regeneration
GFAP-TK	Selective ablation of dividing astrocytes	Ablation of enteric glia Loss of dividing neural stem cells No loss of quiescent astrocytes	Normal behavior and lifespan in absence of CNS injury
Human Tg(GFAP)	Overexpression of human GFAP	Astrocyte hypertrophy, intracellular aggregates (Rosenthal fibers)	None
GLT1 (EAAT2) null	Astrocyte glutamate transporter	Hippocampal neuron loss	Seizures, >50% mortality by 6 weeks
GLAST (EAAT1) null		No significant pathology in absence of injury	Motor incoordination and increased susceptibility to cerebellar cold-induced injury
Connexin 43 (heterozygous)	Gap junction	Reduced connexin 43 expression without significant pathology	Increased stroke volume following middle cerebral artery occlusion
S100B	Calcium–zinc binding protein	No significant pathology	Increased epileptogenesis; altered synaptic plasticity

Abbreviations: GFAP, glial fibrillary acidic protein; Vim, vimentin.

in the CNS, and particularly at the synapse. In mice that are null for the sodium-dependent glutamate transporter GLT1, less than 5% of glutamate transport is preserved. Phenotypically, this mechanism is manifested in the development of seizures accompanied by a reduction in survival in animals after birth. These mice were more prone to acute brain injury, and to an increase in seizure activity and neuronal loss induced by the γ-aminobutyric acid (GABA) antagonist pentylenetetrazole, primarily in the CA1 region of the hippocampus.[18] The susceptibility to neural injury was subsequently highlighted by data documenting baseline elevations in hippocampal CA1 glutamate levels in microdialysate—as well as an abnormal rise in glutamate—in this model following ischemic injury.[19] By contrast, spontaneous seizures were not observed in *GLAST* (*Slc1a3*) knockout mice, but appeared to require a second 'insult'. More-severe episodes of pentylenetetrazole-induced seizure activity were observed in *GLAST* knockout mice when compared with wild-type mice.[20]

Although no mutations have been observed in the coding sequence of the *EAAT2* (*SLC1A2*) gene in humans, loss of EAAT2 protein is seen in neurodegeneration. Mutations in the *EAAT2* promoter are associated with increased serum glutamate and worse outcomes following cerebrovascular insults, and one particular polymorphism in the *EAAT2* promoter is associated with higher glutamate concentrations and higher frequency of progressing stroke.[21]

Ablation of reactive astrocytes: GFAP-TK mice

In addition to cellular hypertrophy and upregulation of specific genes associated with reactive astrocytosis, astrocyte proliferation is also observed following some insults. The GFAP-TK mouse is a transgenic model in which dividing, reactive astrocytes that emerge after CNS injury can be selectively ablated by the administration of ganciclovir. In a stab-and-crush model of spinal cord injury, this ablation of reactive astrocytes resulted in a combination of local tissue disruption, leukocyte infiltration, neuronal and oligodendrocyte death, and motor impairment. These findings indicate that astrocyte proliferation is protective in spinal cord injury, and they demonstrate that the process of proliferation has distinct consequences besides, or in addition to, cellular hypertrophy and upregulation of astrocyte-specific genes.[22]

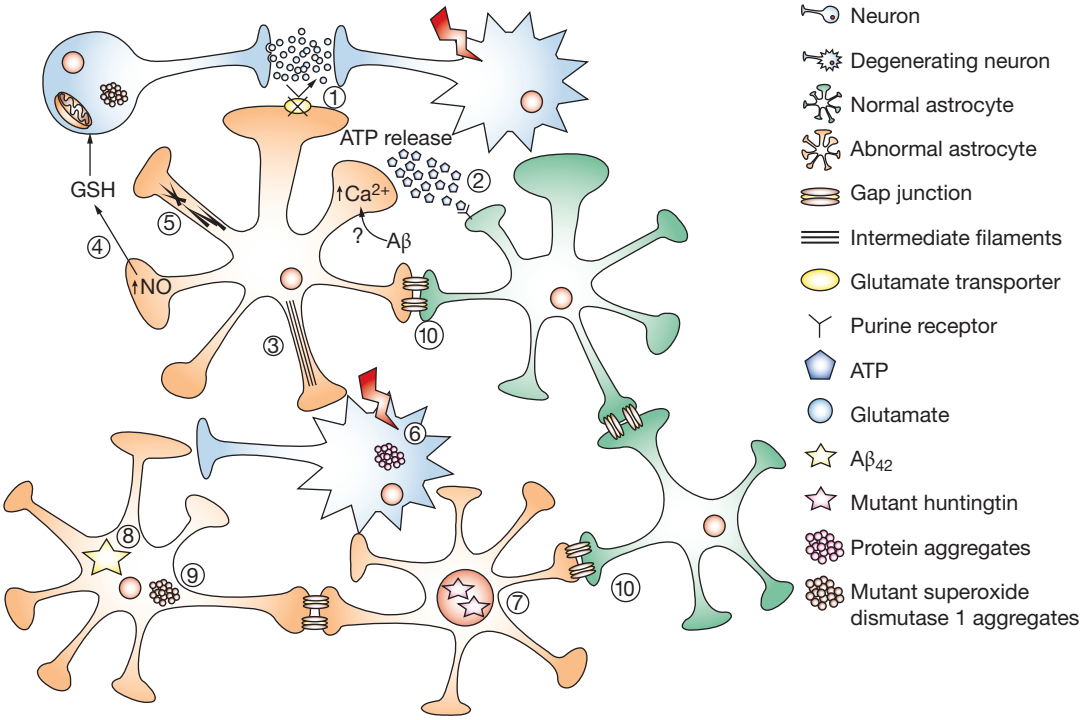

Figure 2 Potential astrocyte dysfunction in neurodegenerative diseases. (1) Impairment of glutamate transporters, through reduced expression, aberrant RNA synthesis or altered function, results in increased synaptic glutamate and excitotoxicity.[64] (2) Amyloid-β could potentially increase the amount of ATP released by astrocytes, as well as interacting with gap junctions to alter calcium signaling and glial communication.[43] (3) Upregulation of glial fibrillary acidic protein is a consistent pathological feature in neurodegenerative diseases, although the significance of this observation is not completely understood.[15] (4) Nitric oxide stimulates the release of glutathione from astrocytes to neurons, thereby increasing neuronal antioxidant reserves and limiting oxidative damage to neuronal mitochondria.[59] (5) Mutations in glial fibrillary acidic protein associated with Alexander disease result in the development of Rosenthal fibers, and lead to disordered intermediate neurofilament organization.[65] (6) Neurons surrounded by mutant astrocytes develop protein aggregates and axonal pathology, and are more susceptible to cell death in several neurodegenerative disease models, including amyotrophic lateral sclerosis, Alzheimer's disease and Huntington's disease.[36,47,56] (7) Mutant huntingtin expressed in astrocytes forms intranuclear aggregates and also influences neuronal cell death *in vitro*.[56] (8) Amyloid-β_{42}-positive material is seen within activated astrocytes in the tissues of individuals with Alzheimer's disease, and is abundant in regions with the most active Alzheimer's disease pathology.[40] (9) Accumulation of mutant superoxide dismutase 1 is also observed in astrocytes.[68] (10) Finally, communication between abnormal or injured astrocytes might affect the biology and function of surrounding normal astrocytes, through either hemichannels or soluble mediators.[25] Abbreviations: Aβ, amyloid-β; GFAP, glial fibrillary acidic protein; GSH, glutathione.

Loss of astrocyte–astrocyte communications: connexin 43 knockout mice

Astrocytes have a unique form of intercellular syncytial communication with neighboring astrocytes through gap junctions, which are primarily composed of Cx43 protein.[23] Studying the role of Cx43 in adult animals has proved to be difficult, because *Cx43* null mice die at birth, probably from cardiopulmonary insufficiency.[24] *Cx43* heterozygous mice have, therefore, been used for *in vivo* studies of this protein. These mice showed a significantly increased stroke volume compared with wild-type mice following middle cerebral artery occlusion.[25] Future studies using this model might help to elucidate the function of Cx43 in neurodegenerative disorders.

ASTROCYTES IN NEURODEGENERATIVE DISEASE

The potential effects of astrocyte dysfunction in neurodegenerative diseases are summarized in Figure 2.

Amyotrophic lateral sclerosis

ALS is the most common form of adult motor neuron disease. This condition is characterized

by progressive degeneration of the upper motor neurons in the cortex and the lower motor neurons in the brainstem and spinal cord. Approximately 95% of reported cases of ALS are apparently sporadic. The remaining ~5% of ALS patients inherit the disease, and this is classified as familial ALS. In 1993, chromosome-21-linked familial ALS was found to be associated with mutations in *SOD1*, the gene that encodes copper–zinc superoxide dismutase;[26] since then, mutations in several other genes, including those encoding dynactin, alsin and senataxin, have been implicated in familial ALS.

Human studies

Common to familial and sporadic ALS is the loss of the astrocyte glutamate transporter EAAT2. Studies of the EAAT2 transporter in tissue from individuals with sporadic ALS showed a marked loss of up to 95% of astroglial EAAT2 protein expression and activity in affected areas of the CNS.[27] A clue to a possible mechanism for EAAT2 reduction or dysfunction was provided by the finding of aberrant *EAAT2* RNA species, which has been implicated in multiple neurodegenerative diseases. The production of truncated EAAT2 protein results in reduced function, and the retention of normal EAAT2 protein within the cytoplasm.[28] The significance of these aberrant *EAAT2* RNA species continues to be debated, however, as they have also been found in some normal controls.[29,30]

Animal models

Transgenic mouse and rat models carrying several *SOD1* mutations have been generated; forms of SOD1 (SODC) protein with the point mutations G93A, G37R and G85R all produce reliable motor neuron degeneration when they are overexpressed in transgenic mice.[31–33] At present, these mouse lines are the most reliable and accurate animal models of ALS, and they have been used extensively in an attempt to understand how mutant SOD1 (mSOD1) causes cell death. As with the human disease, motor neurons are selectively affected, although this selectivity is not absolute—small interneurons also degenerate in the mouse models.

In both human tissue and transgenic models of ALS, there is abundant evidence that astroglial abnormalities and physiological dysfunction precede clinical disease. These changes include reactive astrocytosis that can be seen many months before motor neuron degeneration (G85R),[33] and loss of glutamate transport and GLT1 protein expression before the onset of clinical disease or overt motor neuron degeneration.[34] Is the reduction in GLT1 protein in astrocytes significant? Guo and colleagues addressed this question by overexpressing the EAAT2 protein in astrocytes in the mSOD1 mouse model, and demonstrated an increase in motor neuron survival and a delay in disease onset; similar outcomes are seen with drugs that increase GLT1 expression.[35] This evidence indicates that EAAT2 expressed in astrocytes—and probably also glutamate—influences the timing of disease onset and motor neuron survival.[35] Other changes associated with ALS include increased expression of various proteins in astrocytes, including inducible nitric oxide synthase (iNOS), the copper chaperone CCS, and metallothioneins. Pathologically, early cytosolic proteinaceous aggregates have been found in spinal cord astrocytes from all of the mSOD1 mouse lines examined to date.[26]

Until relatively recently, it was not clear whether the pathological changes observed in astrocytes (and other cells) in the mSOD1 mouse model were in response to initial neuronal injury, or whether mSOD1 in non-neuronal cells (i.e. astrocytes) could influence disease. In chimeric mice, expression of mSOD1 in neurons or motor neurons alone was not sufficient to lead to neuronal death—there had to be concomitant expression in glia. Furthermore, the chimeric wild-type–mSOD1 animals lived longer than nonchimeric mSOD1 mice, to a degree that was proportional to the percentage of wild-type cells present.[36] Another powerful observation from this study was that wild-type motor neurons appeared to undergo degeneration, and developed ubiquitinated inclusions, when surrounded by mSOD1-expressing astroglia. Interestingly, the delivery of small interfering RNA (siRNA) targeting human SOD1 to motor neurons by injection into the muscles of mSOD1 mice and allowing retrograde transport resulted in a delay in (but not complete sparing from) disease onset, and prolongation of survival.[37,38] These findings, although noted to indicate a potential for therapeutic interventions, also highlight the fact that mSOD1 mice still eventually develop disease, and again emphasize a role for other cell types besides motor neurons in ALS pathogenesis.

Alzheimer's disease and the 'tauopathies'

AD is characterized clinically by cognitive loss in two or more domains, including memory, language, calculations, orientation and judgment; the loss must be of sufficient severity to cause social or occupational disability. These clinical features are the result of neuronal death and dysfunction in the cerebral cortex, entorhinal area, hippocampus, ventral striatum and basal forebrain, eventually resulting in severe dementia. Pathologically, the two hallmark findings of the disorder are neurofibrillary tangles and amyloid plaques.[39]

Human studies

In tissue from individuals with AD, activated astrocytes were closely associated with amyloid plaques in the molecular layer of the cerebral cortex.[40] Astrocytes might be activated by human amyloid-β (Aβ),[41] indicating a correlation between this protein and subsequent alterations in astrocyte function. Astrocytes also accumulate neuron-derived amyloid material resulting from local neurodegeneration. Once substantial accumulation of this debris occurs, the astrocytes themselves might undergo cell death, resulting in the formation of $GFAP^+$ amyloid plaques.[42] *In vitro* analyses also indicate that treatment of astrocytes with Aβ results in an increase in calcium-wave signaling between these cells.[43] In cells expressing the familial AD presenilin 1 (*PSEN1*) mutation, calcium oscillations in astrocytes were found to occur at lower ATP and glutamate concentrations than in wild-type astrocytes.[44] These data support a model in which calcium signaling between astrocytes is altered by the disease process, which might, in ways that are not fully understood, contribute to dysfunction or death of neurons.

Animal models

One of the hallmark features of AD and other 'tauopathies' is the accumulation of tau protein in neurons and glia.[45,46] This pattern contrasts markedly with the normal CNS distribution, in which tau is expressed predominantly in axons, and is only expressed at low levels in oligodendrocytes and astrocytes. To assess the contribution of astrocytes to tauopathies, transgenic mice were generated in which the tau protein was expressed selectively in astrocytes. In these mice, there was abundant astrocyte tau pathology associated with neuronal staining of phosphorylated neurofilament epitopes, axon degeneration, and inclusion formation, all of which indicated neuron injury; however, no significant neuronal loss was observed.[47] In an extension of these initial observations, investigators developed transgenic mice overexpressing the tauP301L mutation—which is linked to frontotemporal dementia and parkinsonism (FTDP) in humans—in astrocytes. These mice developed neuromuscular abnormalities with loss of strength. The astrocyte tau pathology was also associated with a reduction in expression and function of the astrocyte-specific glutamate transporters GLT1 and GLAST.[48] The selective tau expression in astrocytes in these models provides more evidence of an astrocyte-mediated effect in models of dementia.

Huntington's disease

HD is a fatal autosomal dominant neurodegenerative disease that results from an expanded DNA segment containing a polymorphic trinucleotide CAG repeat in the gene that encodes the protein huntingtin. The most striking neuropathological changes are gross atrophy of the caudate nucleus and putamen, with concomitant marked neuronal loss and astrogliosis. A selective vulnerability of medium-sized striatal spiny projection neurons with a relative sparing of spiny striatal interneurons is also seen.[49]

Human studies

As with other neurodegenerative disorders, astrocytosis is observed in affected regions of the brain of patients with HD. The huntingtin protein co-localizes with these reactive astrocytes in specific regions.[50] In a small study of three brains from individuals with HD analyzed postmortem, *EAAT2* messenger RNA was reduced in the neostriatum, and the degree of reduction correlated with disease severity. The losses were found to be particularly prominent in the putamen, and less so in the caudate.[51] Other studies have failed to find significant changes in glutamate transport in the brains of patients with HD,[52] and more-conclusive implications for the biology of glutamate transporters in human HD are awaited.

Glutamate excitotoxicity in HD has been hypothesized to result from the failure of astrocytic functions that require cell–cell coupling to maintain their syncytial network and contribute to metabolic homeostasis. Alterations in gap junction expression or uncoupling of gap junctions between astrocytes would cause astrocytes to lose their ability to maintain a proper neuronal environment. In the caudate nucleus (a region with prominent HD pathology), however, Cx43 density was increased with HD, and became

located in patches and accompanied by increased GFAP expression.[53] This observation raises several questions, such as why is there an increase in Cx43 expression?; could this increase result in enhanced astrocyte coupling in an attempt to provide an increased spatial buffer capacity?; and is this a neuroprotective response by astroglia?

Animal models

In a transgenic model of HD, expression of the polyglutamine repeat protein results in a movement disorder with neuronal pathology. A reduction in the messenger RNA levels of GLT1 in the striatum and cortex of these mice was observed, and this was accompanied by a decrease in glutamate uptake. Because downregulation of GLT1 in denervated regions would normally be expected, as described above in experimental models of denervation, the authors were careful to note that the changes in GLT1 expression occurred before any neurodegeneration, thereby potentially implicating GLT1 in part of a neuronal death cascade.[54] Similar findings have been reported in the R6/2 transgenic mouse, which expresses an N-terminal fragment of mutant huntingtin.[55]

Mutant huntingtin protein is known to aggregate in the neurons of transgenic mouse models of HD. Recent evidence, however, indicates that mutant huntingtin is also present in the nuclei of astrocytes, a phenomenon that becomes more robust with age and corresponds with a downregulation of glutamate transporters in these cells. A potential cause-and-effect relationship implicating astrocytes in the neurotoxicity observed in HD was noted following the observation in a neuron–glial co-culture that wild-type glial cells protected neurons against mutant huntingtin-mediated neurotoxicity, whereas glial cells expressing mutant huntingtin increased neuronal vulnerability.[56] Taken together, these observations indicate that mutant huntingtin in glial cells can contribute to neuronal dysfunction and excitotoxicity in HD brains through disorders of astroglial biology.

Parkinson's disease

PD is the second most prevalent neurodegenerative disease, after AD. PD is estimated to affect about 1 million Americans, or about 1% of the population over 60 years of age. PD is caused by the disruption of dopaminergic neurotransmission in the basal ganglia. On pathological examination, the numbers of dopaminergic neurons in the substantia nigra are markedly reduced, and Lewy bodies (cytoplasmic inclusions) are present in the residual dopaminergic neurons.[57] The focus has always been on the loss of these dopamanergic neurons and subsequent depletion of dopamine, but a role for non-neuronal cells in producing neuropathological or neuroprotective functions in PD is becoming increasingly recognized.

Human studies

The studies that have been carried out to date appear to support a neuroprotective role for astrocytes in PD. From pathological examinations, an increase in the number of astrocytes as well as in GFAP expression is observed in PD, as with other neurodegenerative disorders.[58] The pathological evidence indirectly indicates that antioxidant pathways might contribute to this neuroprotective effect, because in control brains the density of glutathione-peroxidase-positive cells was higher in the vicinity of the dopaminergic cell groups known to be resistant to the pathological process of PD. The increase in glutathione-peroxidase-containing cells was inversely correlated with the severity of dopaminergic cell loss in the respective cell groups in patients with PD. The quantity of glutathione-peroxidase-containing cells, therefore, might be critical for a protective effect against oxidative stress.[59] Conversely, the presence of synuclein-positive astrocytes in pathological samples has been shown to correlate with nigral neuronal cell death.[60]

Animal models

What is the timing of astrocytosis in animal models of PD? In a PD model generated by lesioning the brain with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), it appears that astrocytosis occurs after the death of dopaminergic neurons, and that this response remains elevated even after most dopaminergic neurons have died.[61] A more rapid response consisting of increased GFAP immunoreactivity as early as 1 hour following the injection of 6-hydroxydopamine into the nigrostriatal dopamine bundle has been observed, indicating a more direct effect of this compound on astrocytosis. Several pathways for this neuroprotection have been implicated, including the increased activation of astrocytes and neuroprotection in 6-hydroxydopamine models following infusion of interleukin-1β (a cytokine released by activated microglia) into the substantia nigra.[62]

Nitric oxide production and glutathione depletion also appear as consistent features in

human PD. The release of glutathione represents another pathway by which astrocytes might be neuroprotective in PD models. Glutathione production appears to be increased by exposure of astrocytes to nitric oxide, and the increase in glutathione release by astrocytes might increase its availability to neurons, thereby making them less susceptible to reactive nitrogen species. This pattern is consistent with the data in PD patients, in whom glutathione-containing cells are in regions with preserved dopaminergic neurons.[63]

Evidence regarding regulation of glutamate transporter expression and function in PD has been somewhat mixed, with downregulation of glutamate transporters being reported in some studies and upregulation being reported in others. The differences in these studies might be related to the methods by which the lesions were induced.[64]

Alexander disease

Alexander disease is a neurodegenerative disorder that is seen predominantly among the pediatric population. The disorder is associated with a prominent leukoencephalopathy, seizures, and death in the first decade of life. Adult forms have also been described, with ataxia and spasticity. As described above, some of the first suggestions that astrocyte-specific proteins (notably GFAP) were part of the disease pathogenesis came from GFAP overexpression in mouse models. Pioneering work from Brenner, Messing and colleagues subsequently demonstrated GFAP mutations in patients with the disorder.[65] The patients with Alexander disease were heterozygous for the mutations, and none of the parents of these patients carried similar mutations. These data indicate that Alexander disease arises from new mutations in GFAP, and that these mutations act in a dominant fashion. Furthermore, the Rosenthal fibers that are an important pathological observation in Alexander disease place this disease among others, including ALS, AD and PD, in which protein aggregation is a prominent feature.[66]

ASTROCYTES AS POTENTIAL THERAPEUTIC TARGETS

One focus of therapeutics in neurodegenerative disease has been replacement strategies for neurotransmitters, such as levodopa (a dopamine precursor) for PD, or memantine (a glutamate receptor [*N*-methyl-D-aspartic acid] antagonist), for the treatment of AD. A large body of work has been devoted to neuroprotective strategies, with enormous basic science and clinical efforts devoted to treatments that are effective in 'neuronal' cultures. With our increased understanding of the relationship between neurons and glia, however, targeted therapeutics for pathways in which astrocytes have a prominent position, as outlined in this Review, need to be developed. It is also likely that therapeutics that are effective at neuroprotection also enhance astrocyte–neuron interactions, but this possibility has not been fully investigated.

One example of recent efforts to influence astrocyte function in neurodegenerative disease was a clinical trial of ceftriaxone, a third-generation cephalosporin, in ALS. This antibiotic was demonstrated to increase astroglial gene expression and glutamate transporter expression and function in several *in vitro* and *in vivo* models, as well as in transgenic models of chronic neurodegeneration (mSOD1 mouse models).[67]

Future directions might also include modalities to examine astrocyte function *in vivo*. *In vivo* imaging of astrocyte–astrocyte communication (e.g. calcium waves) has already been carried out in living rodents. Tools to examine astrocyte function (e.g. PET imaging ligands) will also be valuable for humans, and are already being developed. With the development of animal models for understanding astrocyte biology and pathways involved in astrocyte dysfunction, new paradigms for the modulation of astroglial function might emerge as therapeutic strategies.

KEY POINTS

- Astrocytes perform critical roles in amino acid, nutrient and ion metabolism in the brain, coupling of neuronal activity and cerebral blood flow, and modulation of excitatory synaptic transmission
- Transgenic and knockout mouse models of astrocyte-specific proteins have demonstrated that astrocytes play a role in both neuroprotection and neurodegeneration, particularly following an insult
- Selective expression of mutant proteins associated with neurodegenerative diseases in astrocytes is sufficient to cause neuronal damage
- Mutations in the astrocyte-specific intermediate filament protein glial fibrillary acidic protein is associated with the neurodegenerative disorder Alexander disease
- Astrocytes might be particularly attractive—and underappreciated—targets for neurodegenerative disease therapeutics

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Competing interests
The authors declared they have no competing interests.