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Glial Fibrillary acidic protein: From intermediate filament assembly and gliosis to neurobiomarker

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Abstract

Glial fibrillary acidic protein (GFAP) is an intermediate filament-III protein uniquely found in astrocytes in the CNS, non-myelinating Schwann cells in the PNS and enteric glial cells. GFAP mRNA expressions are regulated by several nuclear-receptor hormones, growth factors and lipopolysaccharides. GFAP is also subjected to a number of post-translational modifications while GFAP mutations result in protein deposits known as Rosenthal fibers in Alexander disease. GFAP gene activation and protein induction appear to play a critical role in astroglia cell activation (astrogliosis) following CNS injuries and neurodegeneration. Emerging evidence also suggests that, following traumatic brain and spinal cord injuries and stroke, GFAP protein and its breakdown products are rapidly released into biofluids, making them strong candidate biomarkers for such neurological disorders.

GFAP overview and outline

Astrocytes are one type of glial cells in the CNS, a group that also includes resident and perivascular microglia, oligodendrocytes, radial glia and Muller cells. In fact, it is estimated that astroglia cells are the most abundant cell types in the brain, providing both structural and functional support for neurons (including neurotransmitter glutamate recycling and trophic factor release). Astrocytes (astroglia) are characterized by the presence of a unique structural protein, glial fibrillary acidic protein (GFAP) isolated and characterized by Dr. Eng in 1969 [1]. GFAP is known to be present in non-myelinating Schwann cells in the PNS, and the enteric glia cells as part of the enteric nervous system (ENS) [2,3]. In this review article, we will first examine the structural layout of GFAP protein, its various splice variants and the pathological significance of GFAP mutations. We will discuss how GFAP is also regulated both at the transcriptional and the post-translational levels, and how such regulations might impact on GFAP's normal cytoskeletal functions and its involvement in maintaining the activated astroglial cell state (astrogliosis) following nervous system injury. We will further focus on the emerging evidence that GFAP and its modified forms as a

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promising protein biomarker for neurotrauma and stroke. Lastly, we will also discuss how GFAP was identified as a dominant autoantigen following traumatic brain injury (TBI) and its implications in terms of triggering a possible post-TBI and sustained autoimmune response towards the nervous system.

CNS-PNS-ENS specificity

GFAP and protein levels are highly expressed in the CNS (Figure 1)[4,5]- almost exclusively present in its astrocytes. GFAP protein is also present in the PNS along the peripheral nerve fiber track, such as the sciatic nerve. In this case, GFAP is localized to non-myelinating Schwann cells that are believed to be functionally similar to astrocytes [6,7]. In addition, GFAP can be found in the glia cells of the enteric nervous system (ENS) [2,3]. Such sub-epithelial glia cells have a trophic and supporting function of the intestinal epithelial cells and neurons. In addition, as GFAP-bearing glia cells are immediately associated with the enteric neurons and their nerve fibers in the submucosal and muscle layers of the gut, they are perfectly positioned to exert neuromodulation function (for example, by active uptake of extracellular neurotransmitters by glial cell surface neurotransmitter (NT) transporters and subsequent degradation. Enteric glia cell surface also has receptors for various NTs including purine P1 and P2 receptors that are responsive to neuron-released nucleotides and adenosine [8]. Since enteric neurons regulate gut motility and mucosal secretion, GFAP-bearing glial cells are thought to indirectly influence such processes as well. GFAP protein levels in enteric glia cells are also responsive to proinflammatory signals (such as IL-6 or bacterial endotoxin lipopolysaccharide LPS) [9]. Similarly, GFAP (and GDNF) levels are highly upregulated in mucosal plexus in the colon of patients with inflammatory bowel disease (IBD) [10]. In particular, GFAP might be an excellent index of enteric gliosis response to the more severe forms of IBD (such as ulcerative and infectious colitis) over Crohn's disease. Intriguingly, mouse enteric glial can undergo neurogenesis in response to injury [3]. In addition, enteric GFAP levels and phosphorylation are also increased in Parkinson's disease (PD) patients [11]. Further investigation is needed to understand the possible implications of this finding in PD patients.

GFAP protein structure and Function

GFAP is a key intermediate filament (IF) III protein responsible for the cytoskeleton structure of glia cells and for maintaining their mechanical strength, as well as supporting neighboring neurons and the blood brain barrier (BBB) [1]. GFAP is structurally similar to other non-epithelial IF members (class III), including vimentin, desmin, and peripherin, and has a head, rod and tail domains. Activated astrocytes take on the morphology of thickened and elongated processes and GFAP-through its involvement in the IF network- is critical in the maintenance of such structure. In the most abundant isoform, GFAP- α (alpha), the head coil domain is followed by the rod domain that is composed of four coils (1A, 1B, 2A, 2B) which are flanked by three linker regions (1, 1,2 and 2, respectively) (Figure 2A). Such a structural organization is in fact highly conserved among class III IF proteins [12]. Crystal 3-D structure is currently not available, but through homology modeling using vimentin as a template, a 3-D structure of GFAP can be visualized (Figure 2B) [13]. Like other IF proteins, GFAP monomer transition to filamentous form by assembling first into dimers in

parallel formation, followed by antiparallel assembly into tetramers, octamers and so on (Figure 2C) [14]. The N-terminal head domain is critically important for filament elongation (including the M₁ERRRITSARRSY₁₄ motif), while the C-terminal tail domain is also important in facilitating oligomerization [15].

Isoforms /splice variants

GFAP is encoded by a single gene mapped to human chromosome 17q21. To date, there are 10 isoforms/splice variants identified (Figure 3). GFAP- α (Isoform 1) is the predominant isoform in brain and spinal cord, but also presents in PNS [16] and has the classic 432 residues (protein accession # NP_002046.1) with the full usage of the 9 exons within the GFAP gene [17]. GFAP- δ , also called GFAP- ϵ (Isoform 2) (NP_001124491.1) is preferentially expressed by neurogenic astrocytes in the subventricular zone [18-22]. GFAP- δ includes the usage of intron before exon-8 and has alternate C-terminal and 431 residues. This unique C-terminal of GFAP- δ binds specifically to presenilins (PS1, PS2) proteins in yeast-two hybrid screening [20]. Presenilins are also involved in Notch-signaling and may play a critical role committing astrocyte into GFAP+ phenotype or neurogenic phenotype. It is tempting to suggest that presenilins–GFAP- δ interaction might have a regulatory mechanism of the Notch–singling pathway in astrocyte-cell fate determination [20]. Obviously further work is needed to clarify the physiologic and/or pathophysiologic significance of such interaction. Enhanced GFAP- δ/ϵ expression in human astrocytic tumor has been reported [23]. GFAP- κ (kappa) is the third isoform (isoform 3) (NP_001229305.1) including an intron before exon-8 and alternate shorten C-terminal and has 328 residues [24] (2007). GFAP- α , GFAP- ϵ , and GFAP- κ are the three most well characterized isoforms of GFAP. Of interest is that mRNA expression levels of all three isoforms gradually increase during development of the embryonic pig brain [24].

GFAP- β is highly expressed in non-myelinated Schwann cells in the PNS [25]. It includes the usage of sequence before exon-1; the exact start site is currently unknown and has at least 432 residues. mRNA and protein levels of GFAP- β are apparently induced following neural injury [19]. GFAP- γ also includes the sequence before exon-1, but with omitted exon-1 and includes an intron before exon-2. Again, its exact start site is currently unknown, but its length is predicted to be less than 432 residues. GFAP- γ mRNA is enriched in corpus callosum and is also present in bone marrow and spleen [26]. GFAP- ζ (zeta) is formed by the inclusion of the intron before exon-9 and has >438 residues [21] (Figure 3).

There are also four isoforms of GFAP collectively called GFAP+1, reflecting its single nucleotide frame-shift mediated variant formation. They are found in a subset of astrocytes throughout the brain. GFAP+1 includes the GFAP Ex6 with a skipped exon-6 and lacking Coil 2B within the core region (347 residues), GFAP 164 which has shortened exon-6, 7 and lacks Coil 2B (366 residues), GFAP 135 which has shortened exon-6 and lacks Coil 2B (374 residues), and GFAP Ex7 which has skipped exon-7 (418 residues) (Figure 3). The detailed structures of these isoforms are also described by Middeldorp and Hol [17]. Interestingly, in AD brains, astrocytes near amyloid plaques have increased staining of both GFAP- α and GFAP- δ while GFAP+1 were found to be limited to a subset of astrocytes with long processes, with their number increased during the progression of AD [27]. Also, three

GFAP+1 splice forms (GFAP¹³⁵, GFAP^{Ex6} and GFAP¹⁶⁴) can be found in the pyramidal neurons of the hippocampus of AD and Down's syndrome patients [28]. The implication of such neuronal expression of GFAP is still unknown. Lastly, immunohistochemical evidence shows that GFAP^{Ex6} and GFAP¹⁶⁴-positive astrocytes are found elevated and localized in focal lesions associated with chronic epilepsy; however, they appear far out-numbered by the dominant GFAP- α positive astrocytes in the same lesioned regions [29]. Lastly, as many of these isoforms of GFAP have alternative N-terminal (beta, gamma), or alternative (δ/ϵ , κ , ζ) or shortened C-terminal (GFAP+1), it is tempting to suggest that they might have significant effects on GFAP filament assembly (Figure 3).

GFAP Mutations/ SNP and Alexander Disease

GFAP is also a target for a single nucleotide polymorphism (SNP), resulting in Alexander Disease [30-33]. A number of mutations were found mainly in the coding regions of the GFAP gene (L47; C79; H79; E223, H239; A244, R258, C289; D295 & R416), but a few mutations are found in the promoter regions (Table 1, top). These mutations were suggested to be “gain-of-function” mutations [34], as GFAP knockout mice does not duplicate the Alexander Disease phenotype [35,36]. The mutant GFAP proteins show a range of competency in intermediate filament assembly - from (i) fully able to assemble into full length (10 nm) IF, (ii) capable of polymerization but not able to extend to full length IF, to (iii) not able to polymerize at all. In addition, mutated GFAP proteins are more prone to aggregate formation – producing astrocytic inclusions (called Rosenthal fibers) found in Alexander Disease brains [31]. Messing et al. recently reviewed how various GFAP mutations are linked to pathology of Alexander Disease [34]. Some researchers believe GFAP aggregates are toxic to astrocytes – and thus might contribute to the astroglial degeneration and the subsequent white matter degeneration pathology observed in Alexander Disease. Other researchers believe that Rosenthal fiber formation might simply be a reflection of excessive GFAP aggregate accumulation in dysfunctional astrocytes [34] (see Outstanding Questions). Lastly, caspase-cleaved N-terminal GFAP fragment might contribute to GFAP aggregate formation in Alexander Disease [37].

GFAP post-translational modifications

GFAP protein is also subjected to a number of post-translational modifications (PTM) (Table 1, Figure 2). GFAP protein is highly regulated by protein kinases (such as protein kinase-A, calmodulin dependent kinase II and PK-C), with many phosphorylation sites mapped to the N-terminal domain: T7 (PKA), S8 (PKA, PKC, cdk2), S13 (CAMPKII, PK, PKC), S17 (CAMPKII), S38 (CAMPKII, PKA, PKC) and S289 (CAMPKII) (Figure 2; Table 1) [14,38-41]. One of the key phosphorylation pathways of GFAP appears to involve the G-protein-coupled mGluR receptor leading to calcium influx and CAMPKII activation [40,42]. As glutamate is a major excitatory neurotransmitter in the brain, such a mechanism might be involved in the cross-talk between neurons and glial cells [42]. In addition, since most of these phosphorylation sites reside at the N-terminal head domain, phosphorylation of GFAP has a negative effect on filament assembly. GFAP phosphorylation is elevated after hypoxic-ischemia in neonatal pig brain [38].

GFAP was recently identified as endogenously citrullinated, i.e. several arginine residues undergo deamination to citrulline (R30, R36, R270, R406 & R416) [43]. The exact extent of citrullinated GFAP is not fully understood and how it alters GFAP function is not elucidated. However, citrullinated protein epitopes are implicated in evoking autoimmune response [44,45] which could be relevant as GFAP is a dominant autoantigen in certain neurological disorders (*see below*) [5]. A number of lysine residues are also found to be subjected to differential acetylation (K89, K153, K189, K218, K259 & K331) in human spinal cord of amyotrophic lateral sclerosis patients (Figure 2, Table 1) [46]. Such acetylation is spread across the whole GFAP protein, but their effects on GFAP structure and functions are currently unknown and warrant further investigation.

GFAP proteolysis/fragmentation in cultured astrocytes was noted, in some early reports, as mediated by the calcium-activated protease calpain [47,48]. Increases in the fragmented form of GFAP has also been found in the spinal cord of patients with ALS [49]. We recently documented that GFAP is highly vulnerable to calpain-mediated truncation at both the C- and N-terminals. This results in a series of truncated GFAP breakdown product (BDP) (38 to 44 kDa) as compared to the 50 kDa intact protein during glial cell challenge in vitro [50,51] or in experimental brain injury in rodents as well as in human cerebrospinal fluid (CSF) from TBI patients [5,50,51]. These fragments can also be observed in post-mortem human brain preparations (Figure 1). Similarly, we also found that GFAP-BDP can be observed in rodent and human CSF after spinal cord injury [52,53]. The main calpain cleavage sites have been mapped out to be N59-A60 and T383-F384, although there are likely alternative cleavage sites near them. Regardless, the “limit fragment” (~38 kDa) with both N- and C-terminal truncation leaves essentially the rod domain intact but the head and tail domains removed. It is thus predicted that such GFAP-BDP would be unable to assemble into filaments. Caspase can also fragment GFAP in pro-apoptotic conditions, producing a shortened N-terminal fragment in AD brain (about 20 kDa) [54] at the DLTD₂₆₆-A₂₆₇ site, which is located at the beginning of coil 2B (Figure 2). Based on our own data, we also identified a second cleavage site at ELND₇₈*₇₉R between the head and rod domains (Figure 2). Chen et al recently demonstrated that GFAP might be cleaved by caspase-6 and that the C-GFAP (C-terminal GFAP) fragment (24 kDa) is unable to assemble into filaments [37]. The 26 kDa N-GFAP (N-terminal GFAP) can form filamentous structures with variable length but it is prone to form filamentous aggregation [37]. However, in experimental TBI and in human TBI CSF, the calpain mediated GFAP fragments (44-38 kDa) appear to dominate over those produced by caspase [5,50].

The role of GFAP in astrocyte activation (astrogliosis) and GFAP inducers/activators

Astroglial cells respond to brain injury and other neuro-perturbative conditions by undergoing “reactive astrogliosis”, a process whereby astroglial cells undergo cellular hypertrophy (increase of size and protein (GFAP) expression) and proliferation (increase number of glial cells) [1]. TBI itself, and its associated neuroinflammation, cause activation (and proliferation) of astroglia cells into damaged areas and a concomitant increase in GFAP levels [55]. Importantly, since GFAP, together with vimentin, is the key component

responsible for the assembly and extension of the intermediate filament inside the astrocytic processes, it is believed that GFAP induction is critically important for the formation of extended and thickened astrocytic processes observed in reactive gliosis. In fact, an increase in GFAP protein is a prominent feature of TBI and degenerative diseases such as PD and AD [5,56-59]. Different locations around the lesion site may exhibit different severities of gliosis; e.g., a glial scar at the location of damaged tissue may be surrounded by areas with less severe astrocyte proliferation or hypertrophy. Diffused traumatic injury can result in diffuse or more moderate gliosis without scar formation. Activated astrocytes (with highly expressed GFAP) are found to surround amyloid and neurite plaques in AD. Astrocytes in culture can also be used to study glial activation and GFAP induction. It is generally regarded that certain levels of post-injury gliosis might be beneficial to the recovery process following brain injury while excessive gliosis and its associated neuroinflammatory responses will have a negative impact to brain structural and functional recovery. [58]. GFAP knockout mice were found to be essentially normal developmentally. In addition, post-spinal cord injury axonal sprouting and regeneration appear largely unaffected in GFAP^{-/-} mice [35]. These results pointed to possible compensatory mechanism involving the related vimentin protein [35]. However, in a separate study with a peripheral nerve crush model, GFAP^{-/-} mice did show defective Schwann cell differentiation and delayed nerve regeneration [36]. Again this is consistent with the concept that post-injury-GFAP induction and associated reactive gliosis might in fact promote neuroregeneration.

A number of growth factors such as CNTF, FGF and TGF β can induce GFAP gene transcription activation [60,61], leading to increasing GFAP protein levels (Table 1, Figure 4A). Nuclear-receptor hormones (Thyroid hormone, Glucocorticoids) also can activate GFAP transcription [62]. The effect of thyroid hormone might also be mediated via the activation of ROCK pathway [63]. These hormone and growth-factor-based GFAP gene regulators are through to be potentially important in the induction of mature astroglia formation. On the other hand, LPS, via the production of nitric oxide is also a robust activation of cultured glial cells and induction of GFAP gene transcription [64] – it is likely that LPS activates toll-like receptor (TLR-4) and possibly CD14 in astrocytes [65]. Systemic administration or intracerebral injection of LPS also leads to gliosis and upregulation of GFAP protein and neuroinflammation in live animals [66-71]. Thus, LPS-based GFAP protein upregulation is more in line with astrogliosis formation.

GFAP is also modulated endogenously by calcium-dependent binding to EF-hand S100 β . Upon binding, S100 β promotes disassembly of IF [72]. S100 β -binding leads to inhibition of GFAP phosphorylation, which might be the mode of action that led to disassembly of IF [73]. Glial derived neurotrophic factor (GDNF) and neurturin (another glia-derived trophic factor) have also been shown to be glia-protective [74,75]. Thus they might serve as auto-regulating factors that either promote GFAP gene transcriptional activation or stabilize GFAP protein levels (Table 1).

GFAP Suppressors and Glial Targeting Therapeutic Agents

A number of therapeutic agents can inhibit glia cells function or suppress GFAP expression (Table 1). An interesting compound is Prosapide which is a 14-mer (Thr-D-Ala-Leu-Ile-

Asp-Asn-Asn-Ala-Thr-Glu-Glu-Ile-Leu-Tyr) derived from the neurotrophic and glia-tropic N-terminal of human glycoprotein prosaposin [76-78]. Prosaptide is also known to cross BBB to exert its GFAP-suppression effects. Secondly, a number of drug-like agents have reported effects in suppressing either GFAP protein expression and/or gliosis induction. These include Withaferin-A (WF-A), ibudilast, clomipramine, aspirin and curcumin. Withaferin A is a steroidal lactone which binds to and thus inhibits GFAP and the related IF protein vimentin [79,80]. It was first isolated from Ayurvedic medicine Winter cherry and is a cancer drug due to its anti-angiogenesis effects. It was found that Withaferin-A covalently modifies the single Cys-294 of GFAP (and a homologous cysteine residue in vimentin protein) leading to inhibition of IF function of GFAP and filament disassembly [80] (Figure 2, Table 1). WF-A can in fact attenuate GFAP levels and glial cell activation and appears BBB-permeable as it exerts its central effects in a model of retinal gliosis [80]. Ibudilast is an anti-inflammatory drug currently used mainly in Japan as an asthma medicine. Ibudilast is board-spectrum phosphodiesterase inhibitor that also inhibits methamphetamine-induced GFAP upregulation and gliosis and attenuates methamphetamine self-administration and relapses [68,70]. Ibudilast also appears to attenuate mechanical allodynia in rat models of neuropathic pain [81]. Tricyclic antidepressant clomipramine is also found to robustly suppress GFAP levels in vivo [82]. Aspirin/Acetylsalicylic acid (5 mM in vitro), although having various anti-inflammatory actions including inhibition of COX2, was unexpectedly found to also inhibit GFAP up-regulation and glial cell activation via an NF- κ B pathway in vitro [83]. Curcumin might also have beneficial effects by down-regulation of GFAP expression in an in vitro model of Alexander disease [84]. Although the exact modes of action (direct binding or regulation of protein levels) for GFAP attenuation by prosaptide, ibudilast, clomipramine, aspirin and curcumin are presently unknown, they might still be useful in studying the role of GFAP in neuro-disease models. Screening efforts are underway in finding additional druggable molecules that can either specifically suppress or reverse GFAP aggregate formation (for Alexander disease) or suppress GFAP expression (for gliosis), including several novel chemical candidates [76,82]. Molecular modeling and compound docking in silico is also being applied for such drug discovery effort [13]. However, at present it is unclear if binding of small molecule to GFAP monomer is sufficient in (a) reducing the steady state levels of GFAP; (ii) inhibiting GFAP polymerization and thus astrogliosis, or (b) destabilizing intracellular GFAP aggregates (see Outstanding Questions).

GFAP-breakdown product as marker for glial cell injury

Neurotrauma conditions are often associated with neuronal injury or death. However, since astrocytes are a major cell type in the brain we proposed that they are also subjected to mechanical or chemical injury shortly after neurotrauma and in neurodegenerative disorders. Our recent work shows that “glial injury” is a key pathologic event during the acute / subacute phase of neurotrauma (in animal model of TBI, spinal cord injury (SCI), as well as in human TBI/ SCI CSF samples), as aided by our newly identified glial injury signature – i.e. the proteolytic conversion of intact glial protein GFAP (50 kDa) into breakdown products (GFAP-BDPs) of 44-38 kDa by calpain [5,50,52,53,85,86] (Figure 4B). Similarly, GFAP is degraded into GFAP-BDPs by calpain proteases in cultured glial cells challenged

with cytotoxins (staurosporine or calcium ionophore) [51] (Figure 4A). Caspase protease also can contribute to GFAP proteolysis, to a lesser extent (see above) [5].

GFAP as biomarker protein for acute CNS injury and other neurological conditions

Increasing evidence also suggests that GFAP and GFAP-BDP might be a useful tool as biofluid-based marker for a number of neurological conditions. The overall concept is that brain injury causes the release of GFAP-BDP and to a lesser extent, full length GFAP from injured astrocytes to the interstitial fluid (ISF)/extracellular fluid, where these proteins equilibrate into the subarachnoid CSF compartment, then release to the circulating blood by direct venous drainage (glymphatic pathway) ([113] or continue to follow the CSF flow and eventually enter the circulation by diffusing pass the (possibly compromised) brain blood barrier (BBB) (Figure 5). One of the advantages of GFAP as a brain biomarker is that it shows strong brain-specificity and high expression levels to the brain (Figure 1). There are over 1.7 million of TBI cases in the US annually [87]. TBI is categorized by Glasgow comma scale or CT abnormality: severe (GCS 3-8, Cranial computerized tomography (CT) abnormal; ~10%), moderate (GCS 9-12, CT abnormal; ~5-10%) and mild (GCS 13-15, CT normal). Additionally, impact and blast overpressure-wave induced TBI are a signature injury of recent warfare among US military personnel [88,89]. In penetrating TBI as well as overpressure blast-wave induced brain injury in rats, GFAP levels are elevated in CSF and/or serum at 4 to 24 hour after injury [50,90]. Emerging evidence from others and us also shows a robust release of GFAP and its BDPs into CSF and blood compartments hours and even days after the initial severe TBI event [91,92]. GFAP levels are linked to CT pathology and outcome in these subjects. GFAP blood levels can also help predict secondary insults after severe TBI [93]. Since moderate TBI is often difficult to distinguish from mild TBI in emergency room, there are two important studies that show that the blood levels of GFAP and/or its BDPs can be used to predict CT abnormality and thus can potentially differentiate mild vs. moderate TBI [85,86]. Consistent with these reports, blood GFAP loads were also elevated in individuals undergoing breacher training and exposures to blast overpressure waves [94]. GFAP levels in both brain tissue and serum are also elevated in animal model of blast overpressure-wave induced TBI that is combined with psychological stress [95].

There are two type of strokes; hemorrhagic stroke or ischemic stroke. The former is a result of intracerebral hemorrhage, while the later is due to a blockage of major blood vessel, resulting in brain ischemia. It is important to differentiate the two, as tissue plasminogen activator (TPA) is currently the only FDA approved treatment for ischemic stroke. TPA (usually given within 3-4 hours post-injury) works by dissolving the existing blood clots in the brain and reducing further blood clot formation. However, TPA is counter-indicated in cerebral hemorrhage conditions. Increasing evidence shows that GFAP might be a key marker that can distinguish the two. Several studies have shown that GFAP is released within 3-4 hours following hemorrhagic stroke, while its release is delayed to 24-48 h post injury in ischemic stroke [96-100]. Thus, early release of GFAP into blood is a clinically important differentiating indicator of hemorrhagic stroke over ischemic stroke. In addition,

GFAP appears to be useful in tracking progression or outcome of ischemic stroke [97,98] (Figure 5).

Thus these data taken together, strongly suggest that once a robust GFAP sandwich ELISA test become universally available, it will help realize the potential of GFAP as a biomarker for various forms of CNS injury. This is considered an important discovery as to date there are no FDA-approved in vitro diagnostic biofluid tests to monitor brain injury. Thus, it can be envisioned that a GFAP biofluid test being used routinely in brain injury patient management and changing medical practice. In addition, based on others' and our recent observations, GFAP-BDPs formation and their subsequent release (rather than intact GFAP) into biofluids appears intimately tied to astrocyte damage or cell death after brain injury (Figure 5). Thus we reason that the elevated levels of GFAPBDP in accessible biofluids (CSF, blood) could then be used to track the levels and kinetics of astrocyte cell damage following brain trauma – In fact this is an under-investigated area that could be exploited for novel therapy development. In addition, from a clinical standpoint, it will be important to define if there are any differentiating utilities in monitoring intact GFAP vs. GFAP-BDP in biofluid following brain injury (see Outstanding Questions).

GFAP biomarker tests can also be further developed into “theranostic” guide to help much needed drug development for CNS injury as FDA approved therapies in this area are almost non-existing. For instance, drug treatment given after brain injury that are beneficial in reducing brain injury or promoting brain recovery should also reduce the biomarker load [101-103]. GFAP assay might be useful as a neurotoxicity biomarker tool during drug development as well [104]. Additionally, since most glioma (including glioblastoma) also express GFAP, monitoring of biofluid levels of GFAP might reflect such tumor formation or expansion [105,106].

GFAP protein or PTM-form of it might also become an autoantigen in neurodegenerative conditions, autism, workers exposed to lead, or in chronic cerebrovascular disorders [107-110]. We recently reported that about 40% of severe TBI patients unexpectedly show an immunodominant autoantibody response to GFAP and its BDPs 5-6 days post-injury [5]. Our hypothesis is that TBI causes (i) protease-mediated GFAP-breakdown (BDP) product formation in injured glial cells and (ii) subsequent release of such GFAP-BDPs in substantive quantity through the compromised brain-blood barrier into circulation. (iii) The combined effect is that GFAP-BDP becomes accessible to and recognized by the immune system as a non-self-protein, triggering autoantibody response in these vulnerable individuals. (iv) Autoantibody specifically targeting a major brain protein such as GFAP might trigger a persistent autoimmune attack of the CNS and negatively affect TBI patient's long-term outcome. Our findings of GFAP being an post-neurotrauma autoantigen have also been extended to spinal cord injury [52]. It is also possible that citrullinated GFAP are preferentially recognized by the immune system as an autoantigen, as citrullinated proteins are identified as robust autoantigens in autoimmune diseases such as rheumatoid arthritis [44,45] (see Outstanding Questions Box). It is important to further examine if such autoantibody response is long lasting and whether it leads to autoimmune attack of the nervous system, similar to multiple sclerosis. Interestingly, Serum autoantibody to GFAP also might have utility in tracking glioma progression [111].

CONCUSSION AND FUTURE DIRECTION

This review points to the fascinating roles that GFAP is playing in our nervous system. They include maintaining the structure and functions of the GFAP-bearing cells in the CNS, PNS and ENS, while also mediating astroglia cell activation in the event of nervous system injury. It also appears that the number of GFAP splice variants in human is likely to exceed the 10 isoforms identified to date, while the number of GFAP mutations that lead to Alexander disease is still growing. GFAP is also highly regulated both pre- and post-translationally, and is a target of a number of pharmacological agents. Mounting clinical evidence also suggests that GFAP is arguably one of the most promising biofluid-based biomarker with diagnostic, prognostic and even theranostic utilities in managing neuro-injuries and possibly other neuro-diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- Eng LF, et al. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem. Res.* 2000; 25:1439–1451. [PubMed: 11059815]
- Gulbransen BD, Sharkey KA. Novel functional roles for enteric glia in the gastrointestinal tract. *Nature Reviews Gastroenterology & Hepatology.* 2012; 9:625–632. [PubMed: 22890111]
- Laranjeira C, et al. Glial cells in the mouse enteric nervous system can undergo neurogenesis in response to injury. *J. Clin. Invest.* 2011; 121:3412–3424. [PubMed: 21865647]
- Su, A., editor. Dataset: GeneAtlas U133A, gcrma. [Online]. 2012. Available: <http://biogps.org/dataset/1/>. [Accessed: 28-Mar-2015]
- Zhang Z, et al. Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products. *PLoS ONE.* 2014; 9:e92698. [PubMed: 24667434]
- Mokuno K, et al. Neuronal modulation of Schwann cell glial fibrillary acidic protein (GFAP). *J. Neurosci. Res.* 1989; 23:396–405. [PubMed: 2769798]
- Neuberger TJT, Cornbrooks CJC. Transient modulation of Schwann cell antigens after peripheral nerve transection and subsequent regeneration. *J Neurocytol.* 1989; 18:695–710. [PubMed: 2515258]
- Lavoie EG, et al. Ectonucleotidases in the digestive system: focus on NTPDase3 localization. *Am J Physiol Gastrointest Liver Physiol.* 2011; 300:G608–20. [PubMed: 21233276]
- Boyen, von, G.B.T. Proinflammatory cytokines increase glial fibrillary acidic protein expression in enteric glia. *Gut.* 2004; 53:222–228. [PubMed: 14724154]
- Boyen, von, G.B.T., et al. Distribution of enteric glia and GDNF during gut inflammation. *BMC Gastroenterol.* 2011; 11:3. [PubMed: 21235736]
- Clairembault T, et al. Enteric GFAP expression and phosphorylation in Parkinson's disease. *J. Neurochem.* 2014 DOI: 10.1111/jnc.12742.

12. Herrmann H, Aebi U. Intermediate filament assembly: fibrillogenesis is driven by decisive dimer-dimer interactions. *Curr. Opin. Struct. Biol.* 1998; 8:177–185. [PubMed: 9631290]
13. Biswas S, et al. Glial fibrillary acidic protein and related glial proteins as biomarkers of neurotoxicity. 2011; 2:40–50.
14. Rodnight R, et al. Control of the phosphorylation of the astrocyte marker glial fibrillary acidic protein (GFAP) in the immature rat hippocampus by glutamate and calcium ions: possible key factor in astrocytic plasticity. *Braz. J. Med. Biol. Res.* 1997; 30:325–338. [PubMed: 9246230]
15. Ralton JE, et al. Identification of two N-terminal non-alpha-helical domain motifs important in the assembly of glial fibrillary acidic protein. *Journal of Cell Science.* 1994; 107:1935–1948. [PubMed: 7983160]
16. Reeves SA, et al. Molecular cloning and primary structure of human glial fibrillary acidic protein. *Proceedings of the National Academy of Sciences.* 2005; 86:5178–5182.
17. Middeldorp J, Hol EM. GFAP in health and disease. *Progress in Neurobiology.* 2011; 93:421–443. [PubMed: 21219963]
18. Condorelli DF, et al. Structural features of the rat GFAP gene and identification of a novel alternative transcript. *J. Neurosci. Res.* 1999; 56:219–228. [PubMed: 10336251]
19. Condorelli DF, et al. GFAPbeta mRNA expression in the normal rat brain and after neuronal injury. *Neurochem. Res.* 1999; 24:709–714. [PubMed: 10344602]
20. Nielsen AL, et al. A New Splice Variant of Glial Fibrillary Acidic Protein, GFAPepsilon, Interacts with the Presenilin Proteins. *J. Biol. Chem.* 2002; 277:29983–29991. [PubMed: 12058025]
21. Kamphuis W, et al. GFAP isoforms in adult mouse brain with a focus on neurogenic astrocytes and reactive astrogliosis in mouse models of Alzheimer disease. *PLoS ONE.* 2012; 7:e42823–e42823. [PubMed: 22912745]
22. Roelofs RF, et al. Adult human subventricular, subgranular, and subpial zones contain astrocytes with a specialized intermediate filament cytoskeleton. *Glia.* 2005; 52:289–300. [PubMed: 16001427]
23. Choi K-C, et al. Enhanced glial fibrillary acidic protein-delta expression in human astrocytic tumor. *Neuroscience letters.* 2009; 463:182–187. [PubMed: 19647039]
24. Blechinger J, et al. Identification and characterization of GFAPkappa, a novel glial fibrillary acidic protein isoform. *Glia.* 2007; 55:497–507. [PubMed: 17203480]
25. Galea E, et al. Glial fibrillary acidic protein mRNA isoforms: expression in vitro and in vivo. *J. Neurosci. Res.* 1995; 41:452–461. [PubMed: 7473876]
26. Zelenika D, et al. A novel glial fibrillary acidic protein mRNA lacking exon 1. *Brain Res. Mol. Brain Res.* 1995; 30:251–258. [PubMed: 7637576]
27. Kamphuis W, et al. Glial fibrillary acidic protein isoform expression in plaque related astrogliosis in Alzheimer's disease. *Neurobiol. Aging.* 2014; 35:492–510. [PubMed: 24269023]
28. Hol EM, et al. Neuronal expression of GFAP in patients with Alzheimer pathology and identification of novel GFAP splice forms. *Molecular Psychiatry.* 2003; 8:786–796. [PubMed: 12931206]
29. Boer K, et al. Immunohistochemical characterization of the out-of frame splice variants GFAP Delta164/Deltaexon. 6 in focal lesions associated with chronic epilepsy. *Epilepsy Research.* 2010; 90:99–109. [PubMed: 20430588]
30. Quinlan RA, et al. GFAP and its role in Alexander disease. *Experimental cell research.* 2007; 313:2077–2087. [PubMed: 17498694]
31. Hagemann TL, et al. Alexander Disease-Associated Glial Fibrillary Acidic Protein Mutations in Mice Induce Rosenthal Fiber Formation and a White Matter Stress Response. *Journal of Neuroscience.* 2006; 26:11162–11173. [PubMed: 17065456]
32. Prust M, et al. GFAP mutations, age at onset, and clinical subtypes in Alexander disease. *Neurology.* 2011; 77:1287–1294. [PubMed: 21917775]
33. Yoshida T, Nakagawa M. Clinical aspects and pathology of Alexander disease, and morphological and functional alteration of astrocytes induced by GFAP mutation. *Neuropathology.* 2012; 32:440–446. [PubMed: 22118268]

34. Messing A, et al. Alexander Disease. *Journal of Neuroscience*. 2012; 32:5017–5023. [PubMed: 22496548]
35. Wang X, et al. Axonal and nonneuronal cell responses to spinal cord injury in mice lacking glial fibrillary acidic protein. *Exp. Neurol*. 1997; 148:568–576. [PubMed: 9417833]
36. Triolo D, et al. Loss of glial fibrillary acidic protein (GFAP) impairs Schwann cell proliferation and delays nerve regeneration after damage. *Journal of Cell Science*. 2006; 119:3981–3993. [PubMed: 16988027]
37. Chen MH, et al. Caspase cleavage of GFAP produces an assembly-compromised proteolytic fragment that promotes filament aggregation. *ASN Neuro*. 2013; 5:293–308.
38. Sullivan SM, et al. Phosphorylation of GFAP is Associated with Injury in the Neonatal Pig Hypoxic-Ischemic Brain. *Neurochemical research*. 2012; 37:2364–2378. [PubMed: 22528834]
39. Karl J, et al. GFAP phosphorylation studied in digitonin-permeabilized astrocytes: standardization of conditions. *Brain research*. 2000; 853:32–40. [PubMed: 10627305]
40. Kommers T, et al. The mGluR stimulating GFAP phosphorylation in immature hippocampal slices has some properties of a group II receptor. *NeuroReport*. 1999; 10:2119–2123. [PubMed: 10424685]
41. Inagaki M, et al. Glial fibrillary acidic protein: dynamic property and regulation by phosphorylation. *Brain Pathol*. 1994; 4:239–243. [PubMed: 7952265]
42. Pierozan P, et al. The phosphorylation status and cytoskeletal remodeling of striatal astrocytes treated with quinolinic acid. *Experimental cell research*. 2014; 322:313–323. [PubMed: 24583400]
43. Jin Z, et al. Identification and characterization of citrulline-modified brain proteins by combining HCD and CID fragmentation. *Proteomics*. 2013; 13:2682–2691. [PubMed: 23828821]
44. Romero V, et al. Immune-mediated pore-forming pathways induce cellular hypercitrullination and generate citrullinated autoantigens in rheumatoid arthritis. *Science Translational Medicine*. 2013; 5:209ra150–209ra150.
45. György B, et al. Citrullination: A posttranslational modification in health and disease. *The International Journal of Biochemistry & Cell Biology*. 2006; 38:1662–1677. [PubMed: 16730216]
46. Liu D, et al. Proteomic Analysis Reveals Differentially Regulated Protein Acetylation in Human Amyotrophic Lateral Sclerosis Spinal Cord. *PLoS ONE*. 2013; 8:e80779. [PubMed: 24312501]
47. Oh TH, et al. Acidic pH rapidly increases immunoreactivity of glial fibrillary acidic protein in cultured astrocytes. *Glia*. 1995; 13:319–322. [PubMed: 7615340]
48. Lee YBY, et al. Rapid increase in immunoreactivity to GFAP in astrocytes in vitro induced by acidic pH is mediated by calcium influx and calpain I. *Brain research*. 2000; 864:220–229. [PubMed: 10802029]
49. Fujita K, et al. Increases in fragmented glial fibrillary acidic protein levels in the spinal cords of patients with amyotrophic lateral sclerosis. *Neurochemical research*. 1998; 23:169–174. [PubMed: 9475511]
50. Zoltewicz JS, et al. Biomarkers track damage after graded injury severity in a rat model of penetrating brain injury. *J. Neurotrauma*. 2013; 30:1161–1169. [PubMed: 23409698]
51. Guingab-Cagmat J, et al. In vitro MS-based proteomic analysis and absolute quantification of neuronal-glial injury biomarkers in cell culture system. *Electrophoresis*. 2012; 33:3786–3797. [PubMed: 23161537]
52. Yokobori S, et al. Acute Diagnostic Biomarkers for Spinal Cord Injury: Review of the Literature and Preliminary Research Report. *World Neurosurgery* in press. 2015
53. Yokobori, S., et al. Biomarkers in Spinal Cord Injury.. In: Wang, K., et al., editors. *Biomarkers of Brain Injury & Neurological Disorders*. 1st edn. CRC Press; New York: p. 340-354.
54. Mouser PE, et al. Caspase-Mediated Cleavage of Glial Fibrillary Acidic Protein within Degenerating Astrocytes of the Alzheimer's Disease Brain. *Am J. Pathol*. 2006; 168:936–946. [PubMed: 16507909]
55. Tzeng S-F, et al. Prostaglandins and cyclooxygenases in glial cells during brain inflammation. *Curr Drug Targets Inflamm Allergy*. 2005; 4:335–340. [PubMed: 16101543]
56. Lopategui Cabezas I, et al. The role of glial cells in Alzheimer's disease: Potential therapeutic implications. *Neurologia*. 2014; 29:305–309. [PubMed: 23246214]

57. Członkowska A, Kurkowska-Jastrzebska I. Inflammation and gliosis in neurological diseases--clinical implications. *Journal of Neuroimmunology*. 2011; 231:78–85. [PubMed: 20943275]
58. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in neurosciences*. 2009; 32:638–647. [PubMed: 19782411]
59. Yu I, et al. Glial cell-mediated deterioration and repair of the nervous system after traumatic brain injury in a rat model as assessed by positron emission tomography. *J. Neurotrauma*. 2010; 27:1463–1475. [PubMed: 20504160]
60. Levison SW, et al. Ciliary neurotrophic factor stimulates nuclear hypertrophy and increases the GFAP content of cultured astrocytes. *Brain research*. 1998; 803:189–193. [PubMed: 9729376]
61. Levison SW, et al. Acute exposure to CNTF in vivo induces multiple components of reactive gliosis. *Exp. Neurol*. 1996; 141:256–268. [PubMed: 8812159]
62. Gomes FC, et al. Glial fibrillary acidic protein (GFAP): modulation by growth factors and its implication in astrocyte differentiation. *Braz. J. Med. Biol. Res*. 1999; 32:619–631. [PubMed: 10412574]
63. Zamoner A, et al. Thyroid Hormones Reorganize the Cytoskeleton of Glial Cells Through Gfap Phosphorylation and RhoA-Dependent Mechanisms. *Cell Mol Neurobiol*. 2007; 27:845–865. [PubMed: 17334943]
64. Brahmachari S, et al. Induction of glial fibrillary acidic protein expression in astrocytes by nitric oxide. *Journal of Neuroscience*. 2006; 26:4930–4939. [PubMed: 16672668]
65. Tarassishin L, et al. LPS and IL-1 differentially activate mouse and human astrocytes: role of CD14. *Glia*. 2014; 62:999–1013. [PubMed: 24659539]
66. Snider SE, et al. Glial cell modulators attenuate methamphetamine self-administration in therat. *Eur. J. Pharmacol*. 2013; 701:124–130. [PubMed: 23375937]
67. Kumar A, Loane DJ. Neuroinflammation after traumatic brain injury: Opportunities for therapeutic intervention. *Brain Behav. Immun*. 2012; 26:1191–1201. [PubMed: 22728326]
68. Johnson KW, et al. Ibudilast for the treatment of drug addiction and other neurological conditions. *Clinical Investigation*. 2014; 4:269–279.
69. Noh H, et al. Systemic injection of LPS induces region-specific neuroinflammation and mitochondrial dysfunction in normal mouse brain. *Neurochem. Int*. 2014; 69:35–40. [PubMed: 24607701]
70. Beardsley PM, et al. The glial cell modulator and phosphodiesterase inhibitor, AV411 (ibudilast), attenuates prime- and stress-induced methamphetamine relapse. *Eur. J. Pharmacol*. 2010; 637:102–108. [PubMed: 20399770]
71. Beurel E, Jope RS. Lipopolysaccharide-induced interleukin-6 production is controlled by glycogen synthase kinase-3 and STAT3 in the brain. *Journal of Neuroinflammation*. 2009; 6:9. [PubMed: 19284588]
72. Bianchi R, et al. S-100 protein, but not calmodulin, binds to the glial fibrillary acidic protein and inhibits its polymerization in a Ca(2+)-dependent manner. *J. Biol. Chem*. 1993; 268:12669–12674. [PubMed: 8509402]
73. Frizzo JK, et al. S100B-mediated inhibition of the phosphorylation of GFAP is prevented by TRTK-12. *Neurochem. Res*. 2004; 29:735–740. [PubMed: 15098935]
74. Uzdensky A, et al. Protection Effect of GDNF and Neurturin on Photosensitized Crayfish Neurons and Glial Cells. *Journal of molecular neuroscience*. 2012; 49:480–490. [PubMed: 22847529]
75. Harvey BK, et al. Stroke and TGF- β proteins: glial cell line-derived neurotrophic factor and bone morphogenetic protein. *Pharmacology & Therapeutics*. 2005; 105:113–125. [PubMed: 15670622]
76. Messing A, et al. Strategies for treatment in Alexander disease. *Neurotherapeutics*. 2010; 7:507–515. [PubMed: 20880512]
77. Taylor EM, et al. Retro-inverso prosaptide peptides retain bioactivity, are stable In vivo, and are blood-brain barrier permeable. *J. Pharmacol. Exp. Ther*. 2000; 295:190–194. [PubMed: 10991978]
78. Meyer RC, et al. GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. *Proceedings of the National Academy of Sciences*. 2013; 110:9529–9534.

79. Bargagna-Mohan P, et al. The tumor inhibitor and antiangiogenic agent withaferin A targets the intermediate filament protein vimentin. *Chemistry & Biology*. 2007; 14:623–634. [PubMed: 17584610]
80. Bargagna-Mohan P, et al. Withaferin A Targets Intermediate Filaments Glial Fibrillary Acidic Protein and Vimentin in a Model of Retinal Gliosis. *J. Biol. Chem*. 2010; 285:7657–7669. [PubMed: 20048155]
81. Ellis A, et al. Systemic administration of propentofylline, ibudilast, and (+)- naltrexone each reverses mechanical allodynia in a novel rat model of central neuropathic pain. *J Pain*. 2014; 15:407–421. [PubMed: 24412802]
82. Cho W, et al. Drug screening to identify suppressors of GFAP expression. *Human Molecular Genetics*. 2010; 19:3169–3178. [PubMed: 20538881]
83. Bae M-K, et al. Aspirin-induced blockade of NF-kappaB activity restrains up-regulation of glial fibrillary acidic protein in human astroglial cells. *Biochim. Biophys. Acta*. 2006; 1763:282–289. [PubMed: 16516312]
84. Bachetti T, et al. Beneficial effects of curcumin on GFAP filament organization and down-regulation of GFAP expression in an in vitro model of Alexander disease. *Experimental cell research*. 2012; 318:1844–1854. [PubMed: 22705585]
85. Papa L, et al. Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. *Ann Emerg Med*. 2012; 59:471–483. [PubMed: 22071014]
86. Okonkwo DO, et al. GFAP-BDP as an acute diagnostic marker in traumatic brain injury: results from the prospective transforming research and clinical knowledge in traumatic brain injury study. *J. Neurotrauma*. 2013; 30:1490–1497. [PubMed: 23489259]
87. Gerberding, JL., et al. Report to Congress on Mild Traumatic Brain Injury in the United States: Steps to Prevent a Serious Public Health Problem. Atlanta, GA.: 2003.
88. Martin EM, et al. Traumatic brain injuries sustained in the Afghanistan and Iraq wars. *Am J Nurs*. 2008; 108:40–7–. 47–8. quiz. [PubMed: 18367927]
89. Jaffee DCMS, Meyer KS. A brief overview of traumatic brain injury (TBI) and post-traumatic stress disorder (PTSD) within the Department of Defense. *The Clinical Neuropsychologist*. 2009; 23:1291–1298. [PubMed: 19882472]
90. Svetlov SI, et al. Neuro-Glial and Systemic Mechanisms of Pathological Responses to Primary Blast Overpressure (OP) Compared to Severe “Composite” Blast in Rat Models. NATO Research and Technology Organisation. RTO-MP-HFM. 2012; 207:37–1 –37–10.
91. Mondello S, et al. Glial neuronal ratio: a novel index for differentiating injury type in patients with severe traumatic brain injury. *J. Neurotrauma*. 2012; 29:1096–1104. [PubMed: 22165978]
92. Vos PE, et al. GFAP and S100B are biomarkers of traumatic brain injury: an observational cohort study. *Neurology*. 2010; 75:1786–1793. [PubMed: 21079180]
93. Stein DM, et al. Use of serum biomarkers to predict cerebral hypoxia after severe traumatic brain injury. *J. Neurotrauma*. 2012; 29:1140–1149. [PubMed: 22360297]
94. Tate CM, et al. Serum Brain Biomarker Level, Neurocognitive Performance and Self-reported Symptom Changes in Soldiers Repeatedly Exposed to Low-Level Blast: A Breacher Pilot Study. *J. Neurotrauma*. 2013 DOI: 10.1089/neu.2012.2683.
95. Kwon SK, et al. Stress and traumatic brain injury: a behavioral, proteomics, and histological study. *Front Neurol*. 2011; 2:12. [PubMed: 21441982]
96. Herrmann M, et al. Release of glial tissue-specific proteins after acute stroke: A comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. *Stroke*. 2000; 31:2670–2677. [PubMed: 11062293]
97. Zhang J, et al. Serum glial fibrillary acidic protein as a biomarker for differentiating intracerebral hemorrhage and ischemic stroke in patients with symptoms of acute stroke: a systematic review and meta-analysis. *Neurol. Sci*. 2013; 34:1887–1892. [PubMed: 24036689]
98. Wunderlich MT, et al. Release of glial fibrillary acidic protein is related to the neurovascular status in acute ischemic stroke. *Eur J Neurol*. 2006; 13:1118–1123. [PubMed: 16987165]
99. Foerch C, et al. [Glial fibrillary acidic protein in patients with symptoms of acute stroke: diagnostic marker of cerebral hemorrhage]. *Nervenarzt*. 2014; 85:982–989. [PubMed: 25057113]

100. Foerch C, et al. Serum glial fibrillary acidic protein as a biomarker for intracerebral haemorrhage in patients with acute stroke. *Journal of Neurology, Neurosurgery & Psychiatry*. 2006; 77:181–184.
101. Wagner AK, Zitelli KT. A Rehabilomics focused perspective on molecular mechanisms underlying neurological injury, complications, and recovery after severe TBI. *Pathophysiology*. 2013; 20:39–48. [PubMed: 22444246]
102. Kochanek PM, et al. A novel multicenter preclinical drug screening and biomarker consortium for experimental traumatic brain injury: operation brain trauma therapy. *J Trauma*. 2011; 71:S15–S24. [PubMed: 21795873]
103. Zhang Z, et al. Systems biology and theranostic approach to drug discovery and development to treat traumatic brain injury. *Methods Mol. Biol.* 2010; 662:317–329. [PubMed: 20824479]
104. O'Callaghan JP, Sriram K. Glial fibrillary acidic protein and related glial proteins as biomarkers of neurotoxicity. *Expert Opin. Drug Saf.* 2005; 4:433–442. [PubMed: 15934851]
105. Jung CS, et al. Serum GFAP is a diagnostic marker for glioblastoma multiforme. *Brain*. 2007; 130:3336–3341. [PubMed: 17998256]
106. Brommeland T, et al. Serum levels of glial fibrillary acidic protein correlate to tumour volume of high-grade gliomas. *Acta Neurologica Scandinavica*. 2007; 116:380–384. [PubMed: 17986096]
107. Moneim IA, et al. Autoantibodies to neurofilaments (NF), glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) in workers exposed to lead. *The Journal Of The Egyptian Public Health Association*. 1999; 74:121–138. [PubMed: 17216956]
108. Poletaev ABA, et al. Serum anti-S100b, anti-GFAP and anti-NGF autoantibodies of IgG class in healthy persons and patients with mental and neurological disorders. *Autoimmunity*. 2000; 32:33–38. [PubMed: 10958173]
109. Kamchatnov PR. Autoantibodies to GFAP (glial fibrillary acidic protein) and to dopamine in patients with acute and chronic cerebrovascular disorders. *Health*. 2010; 2:1366–1371.
110. Ishida K, et al. Identification and characterization of an anti-glial fibrillary acidic protein antibody with a unique specificity in a demented patient with an autoimmune disorder. *Journal of the Neurological Sciences*. 1997; 151:41–48. [PubMed: 9335009]
111. Wei P, et al. Serum GFAP autoantibody as an ELISA-detectable glioma marker. *Tumor Biol*. 2013; 34:2283–2292.
112. Zhang Z, et al. Multiple alphaII-spectrin breakdown products distinguish calpain and caspase dominated necrotic and apoptotic cell death pathways. *Apoptosis*. 2009; 14:1289–1298. [PubMed: 19771521]
113. Plog BA, et al. Biomarkers of Traumatic Injury Are Transported from Brain to Blood via the Glymphatic System. *Journal of Neuroscience*. 2015; 35:518–526. [PubMed: 25589747]

Highlight

GFAP is tightly regulated at mRNA level and by post-translational modifications

GFAP plays a critical role in astrogliosis after CNS injury and in neurodegeneration

GFAP as a potential drug target for Alexander's disease and neurodegeneration

GFAP and breakdown products are emerging biomarkers for TBI and neuro-injuries

Outstanding Questions Box

Outstanding Questions

- Does GFAP citrullination and/or acetylation plays a role in evoking autoimmune response to GFAP after brain injury or in other neurological disorders?
- What is the exact mechanistic relationship between aggregates of GFAP (Rosenthal fibers), astrocytic degeneration and white matter degeneration in Alexander disease?
- How might the expression of different isoforms of GFAP produce different astrocyte responses to neuroinjury and/or other neuro-perturbation?
- What are the Differentiating utilities in monitoring intact GFAP vs. GFAP-BDP in biofluid following brain injury [e.g. Do the CSF or blood levels of GFAP-BDP better reflect the extent of astrocyte injury post-TBI than the levels of intact GFAP protein]?
- If specific GFAP-binding or GFAP- inhibiting pharmacological agents can be developed, will they be useful in treating neuroinjury conditions or other neurological disorders (e.g. Alexander's disease)?

Figure 1. GFAP tissue specificity
(A) GFAP mRNA expression in human tissue and cells based on BioGPS database [4] (B) GFAP protein expression in various human and rat organs, tissues Human GFAP was extensively truncated to 48-38 kDa bands, likely due to postmortem proteolysis, while rat GFAP mainly exists in brain as 50-48 kDa form [5].

Figure 2. Glial Fibrillary Acidic Protein (GFAP) structure and assembly
 (A) linear structure, functional domains and key modifications. (B) 3D-GFAP protein structure based on Ref. [13]; (C) Proposed GFAP dimer and tetramer assembly and oligomerization model, modified after Ref. [14].

GFAP isoforms human accession no.		Localization	a.a.	Features
Gfap-α (Isoform 1) NP_002046.1		-Main isoform in astrocytes in CNS, -Also in Schwann cells PNS	432	Predominant isoform in brain
Gfap-β		-high levels in PNS- Schwann cells	>432	-Included sequence before exon-1; -Exact start site unknown
Gfap-γ		-Corpus callosum -mRNA found in brain, bone marrow, spleen	<432	-Included sequence before exon-1, omission of exon-1; included intron before exon-2 -exact start site unknown
Gfap-δ /GFAP-ε (Isoform 2) NP_001124491.1		-Preferentially expressed by neurogenic astrocytes (SVZ) -Potential binding to Presenilins	431	-included intron before exon-8, alternate C-terminal
Gfap-κ (kappa) (Isoform 3) NP_001229305.1		-Transcripts can be found in mouse cortex, striatum and cerebellum	438	-included intron before exon-8; alternate C-terminal
Gfap-ζ (zeta)		-Newly identified isoform	>432	Included intron before exon-9
GFAP +1	GFAPΔEx6	-Collectively called GFAP+1 reflecting its frameshift mediated variant formation -Subset of astrocytes throughout the brain; -- Implicated in AD, Downs, epilepsy	347	Skipped exon-6; lacking Coil 2B -Exact start site unknown
	GFAPΔ164		366	-Shortened exon-6, 7; lacking Coil 2B -Exact start site unknown
	GFAPΔ135		374	-Shortened exon-6; lacking Coil 2B
	GFAPΔEx7		418	-Skipped exon-7 -Exact start site unknown

Figure 3. Known GFAP isoforms and features
(Left) Description of key features of GFAP isoforms. (Right) Schematic comparison of key GFAP isoforms in linear models.

Figure 4. GFAP patterns in injured and activated glia cells in vitro and in vivo

(A) Rat GFAP staining of resting primary glia cells or Staurosporine (0.5 μ M, 5 h)-injured glia or CNTF (200 nM for 24 h)- activated glial cells. (B) Naïve (control) rat cortex or 24 h after experimental TBI showing injured glia cells or 3 day after TBI showing activated glia cells. GFAP was stained red, while counterstain was DAPI for nuclear DNA (blue). (C) Anti-GFAP-immunoblotting of control glia cell lysate shows intact GFAP of 50 kDa with two anti-GFAP antibodies. Upon induction of necrosis (induced by 10 μ M A23187) or caspase-dominant apoptosis (induced by 5 mM EDTA) [51,112], core-directed antibody shows calpain-mediated 38 kDa limit GFAP-BDP after A23187 treatment, while C-terminal antibody detects two distinct caspase-mediated GFAP-BDPs of 44K and 20K after EDTA treatment (Results shown in both (A) and (B) are from a published study by us [5], but were not shown as representative examples in a primary publication).

Neuro-Disease/ Disorder	Species, Biofluid type	Temporal Profile	Key Refs.
Hemorrhagic stroke; ICH	Human serum	< 4 h	[96-100]
Cerebral ischemia	Human ischemic stroke Rat MCAO	24-72 h	[97-98]
Severe TBI	Human CSF serum Rat CSF, serum	6 h – 24 h	[90-93]
Mild TBI	human serum, plasma	< 24 h	[85-86]
Spinal cord injury	Human, CSF, serum Rat CSF, serum	< 24 h	[52-53]
Glioma / Glioblastoma	Human biofluid	-	[105-106]

Figure 5. Release of GFAP and GFAP-BDP into biofluid as acute CNS injury biomarker (Left) List of neuro-diseases and disorders in which GFAP and/or GFAP-BDP is released into biofluid. (Right) Schematic showing how GFAP BDP is generated after TBI and how both GFAP-BDP and to a lesser extent intact GFAP are released into interstitial fluid (ISF)/ extracellular fluid. From there, these proteins diffused into the subarachnoid CSF [113]. GFAP-BDP/GFAP then either drain directly into the veins (glymphatic pathway, dashed arrows) or continue to follow the CSF flow through the ventricles and then enter the circulation by diffusing through the BBB. Blood-based GFAP and GFAP-BDP can be detected as a biomarker, but they also can serve as autoantigen, triggering autoantibody response in a subset of patients.

Table 1

GFAP modifications and modulators

<i>Known modifications of GFAP</i>			
Modification	Site	Effects	Key Refs
Phosphorylation	T7(PKA) S8 (PKA, PKC, cdk2) S13 (CAMPKII, PKA, PKC) S17 (CAMPKII) S38 (CAMPKII, PKA, PKC) S289 (CAMPKII)	Inhibition of GFAP polymerization	[38-42]
Citrullination	Arginine deimination to citrulline at R30, R36, R270, R406 & R416	Altered GFAP confirmation; increased autoimmune response	[43]
Acetylation	Acetylated lysine residues at K89, K153, K189, K218, K259 & K331	Found in ALS spinal cord (currently unknown effects of acetylation)	[46]
Proteolysis	Calpain sites (major): 69-70; 383-384 (BDP of 44K-38K) Caspase sites : 78-79, 266-267 (BDPs of 30K, 20K)	Disruption of IF elongation BDP appears glia-toxic	[47-54]
SNP (Single nucleotide polymorphism)	Promoter region; L47; C79; H79; E223, H239; A244, R258, C289; D295 & R416	Formation of GFAP mutant aggregates (Rosenthal fibers) and causing Alexander Disease	[32-34]

<i>GFAP modulators</i>			
Modulating molecule	Chemical class	Mode of action & effects	Key Ref.
<i>Inducers</i>			
Thyroid hormone, Glucocorticoids	Nuclear-receptor hormones	GFAP gene transcriptional activation	[62-63]
CNTF, (FGF and TGF-β1)	Growth factors	GFAP gene transcriptional activation	[60-61]
Lipopolysaccharide (LPS)	Bacteria endotoxins	GFAP gene transcriptional activation	[65,69]
GDNF and neurturin	glia-derived trophic factor	Promote GFAP gene transcriptional activation or stabilize GFAP protein levels	[74-75]
<i>Suppressors/ Inhibitors</i>			
S100b	Glial protein	Binds and inhibits GFAP phosphorylation, promote disassembly of IF	[72-73]
Withaferin-A	Steroidal lactone (from winter cherry)	GFAP-covalent modification of Cys-294; inhibition of IF function	[79-80]
Prosaptide	N-terminal of prosaposin	Down-regulation of GFAP expression (<i>precise mode of action unknown</i>)	[76-78]
Ibudilast (AV411)	Pan PDE inhibitor	Down-regulation of GFAP expression (<i>precise mode of action unknown</i>)	[68, 70, 81]
Clomipramine (Clofranil)	Tricyclic antidepressant	Down-regulation of GFAP expression (<i>precise mode of action unknown</i>)	[82]
Aspirin/Acetylsalicylic acid	Cox-1 inhibitor	Down-regulation of GFAP expression (5 mM in vitro) (<i>precise mode of action unknown</i>)	[83]
Curcumin	Curcuminoid (from turmeric)	Down-regulation of GFAP expression (<i>precise mode of action unknown</i>)	[84]