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REVIEWS

Glial Fibrillary Acidic Protein (GFAP): on the 45th Anniversary of Its Discovery

A. A. Tykhomyrov,[1] A. S. Pavlova,[1] and V. S. Nedzvetsky[2]

We dedicate this review to the blessed memory of Professor Volodymyr O. Berezin (1946-2016), the founder of the innovative Ukrainian school that joined efforts of researchers in order to study astroglia and its specific proteins.

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Glial fibrillary acidic protein (GFAP) is the main component of intermediate filaments of the cytoskeleton of astrocytes. Over more than four decades of fundamental and applied studies, GFAP achieved the status of the classical marker for astroglia. Our review deals with the analysis and systematization of the literature data describing the peculiarities of the structural organization of the molecules of this protein, its isoform composition, and changes in expression of the *GFAP* gene in the course of CNS ontogenesis; the hierarchical principle of the formation of glial intermediate filaments is also described. A great deal of information about key reactions of post-translational modifications of GFAP and their role in the functioning of the above-mentioned protein is conveyed. Based on the modern literature data, the limited proteolysis of GFAP is considered not only a stage of catabolic transformation of this protein but also a mechanism underlying regulation of the dynamic properties of the cytoskeleton in astroglial cells. It is believed that the main functions of GFAP are the maintenance of specific morphology of astrocytes, control of migration of these cells, and maintenance of the stability of their processes; however, more and more findings are indicative of the involvement of this protein in the processes of cellular signalling and modulation of neuron-to-glia interactions. GFAP as a component of intermediate filaments of the cytoskeleton plays a key role in the development of reactive astrocytosis, i.e., of a typical response of the CNS to injury. Overexpression of GFAP or suppression of its biosynthesis reflect modifications of the functional activity of astrocytes related to damage to the nerve tissue, metabolic abnormalities, and development of neurodegenerative states. Quantitative estimation of GFAP and of its breakdown products, as well as that of anti-GFAP autoantibodies in biological fluids, are at present used as significant criteria in the diagnostics of neurodegenerative pathologies. Non-canonical functions of GFAP, which it fulfills in non-astrocyte units, are indicative of functional polymorphism of this protein and need further investigations.

Keywords: glial fibrillary acidic protein (GFAP), astrocytes, cytoskeleton, intermediate filaments (IFs), reactive astrocytosis.

INTRODUCTION

Glial fibrillary acidic protein (GFAP) is a monomer protein subunit of intermediate filaments (IFs) of the cytoskeleton of astrocytes. Taking into consideration that some cells of other histotypes are capable of synthesizing GFAP, but only in limited amounts, this protein is used as a rather specific molecular marker for astroglia. GFAP belongs to class III of IF proteins, which also includes vimentin, desmin, and peripherin [1, 2]. Discovery of GFAP by Eng in the early 70s of the 20th Century marked the beginning of a new era in studies of neurophysiology of glia [3]. Just due to the discovery of this astrocytic marker and to obtaining

[1] Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine.
[2] Oles' Gonchar Dnipropetrovsk National University, Dnipropetrovsk, Ukraine.
Correspondence should be addressed to A. A. Tykhomyrov (e-mail: artem_tykhomyrov@ukr.net).

the corresponding antibodies, examination of intracellular processes in astrocytes in detail and visualization and estimation of the functional state of these star-shaped cerebral cells were initiated and developed. The first studies of cellular functions of GFAP-containing filaments contributed to the formation of a paradigm of their leading role in the modulation of mobility of astrocytes, specificity of morphology of these cells, and stability of the astrocytic processes [4, 5]. However, the concept on the functional importance of the above-mentioned structures was expanded significantly; researchers came to perceive that the role of GFAP in the functioning of astrocytes is not limited exclusively by the structural/integrative aspect. Unique physico-chemical properties of GFAP define the ability of its polymers to associate with other cytoskeletal components; in this case, different protein-to-protein interactions are controlled at the level of post-translational modifications [6, 7]. Molecular cloning of the *GFAP* gene in 1985 opened up new prospects for investigation of the functional specificities of this protein [8]. Cloning of *GFAP* gene-knockout animals and other molecular/genetic approaches showed that astrocytic IF protein is an active modulator of some processes, including neuron-to-glia communication, modulation of synaptic transmission, myelination of nerve fibers, formation of the architecture of the white matter, and maintenance of the integrity of the blood brain barrier (BBB) [1, 2, 6].

After injuries induced by traumas, neurodegenerative diseases, genetic disorders, and/or chemical intoxication, astrocyte units in the CNS of vertebrates demonstrate certain clearly pronounced reactions; such a typical response was called *astrocytosis* [9, 10]. In reactive astrocytes, intense proliferation, hypertrophy, excessive expression of GFAP, and intensified fibrillogenesis are observed. Accumulation of reactive astrocytes, whose cellular somata and processes are filled with GFAP-containing filaments, forms a protective structure (glial cicatrix) in the nerve tissue. It has been proved that variations of the profile of GFAP expression, as well as the intensity of its catabolic transformations, correlate with changes in the functional activity of astrocytes [11, 12]. Based on these data, it has been believed that GFAP is a powerful and informative index of a wide range of neurodegenerative disorders caused by different unfavorable influences or diseases [13]. Immunochemical detection of GFAP is used for identification of the type of neoplasms of a glial origin [14]. The data obtained in the course of the analysis of autoantibodies against GFAP in biological fluids of the organism are of a great diagnostic value for identification of autoimmune astrocytopathies and estimation of the level of severity of traumatization of the nerve tissue [15].

Over the past decades, examination of this protein created a howling sensation, which was caused by the necessity for clarification of a wide range of issues related to investigation of the structural/functional peculiarities of GFAP and prospects of the applied using of the obtained data. The number of publications devoted to the corresponding problems reaches at present about 30,000 and is rapidly increasing (Table 1). Unfortunately, over the past years in Ukraine there is a certain deficit of published papers aimed at generalization of the respective actual data. Therefore, we tried to systemize and critically analyze in our review the available data and our own findings with respect to localization and properties of GFAP. We consider the structural peculiarities of the GFAP molecule, synthesis of this protein, principles of the assemblage of filaments, metabolism of GFAP (including its covalent post-translational modifications and proteolysis), functions of GFAP within the astrocytic cytoskeleton, the role of this

T A B L E 1. Number of Citations Related to the Terms “Glial Fibrillary Acidic Protein” or “GFAP” in PubMed Search on Request

Period (years)	“Glial fibrillary acidic protein”	“GFAP”	In total
2011-2016	4358…	3842…	8200…
2010-2006	3911	2698	6609
2005-2001	3403	1919	5322
2000-1996	2664	1577	4241
1995-1991	2398	1359	3757
1990-1986	1565	850	2415
1985-1981	714	278	992
1980-1972	116	23	139

protein in the development of reactive astrocytosis, application of GFAP as a marker for injured nerve tissues, and peculiarities of expression and functioning of GFAP beyond astrocytes.

HYSTORY OF DISCOVERY OF GFAP

This protein was for the first time isolated in 1969 by Eng et al. [16] from cerebral tissues of patients with multiple sclerosis; the authors described this protein as a *plaque protein* [16]. Later on, GFAP was identified as one of the main components present in the cerebral parts of patients suffering from a severe form of fibrous gliosis. Such parts of the brain looked like accumulations of fibrous astrocytes and demyelinated neurons. In the article published in 1971 in *Brain Research*, the characteristics of the above protein and the technique for its isolation from a fraction of water-insoluble proteins of sclerotic plaques were described in detail [3]. The final name of this protein came to be known as GFAP from 1972 [5]. In the same year, specific antibodies against GFAP were obtained for the first time [4].

LOCALIZATION OF GFAP

In the CNS, GFAP is synthesized exclusively by astrocytes and is the most familiar protein of these cells. The cellular specificity of biosynthesis of GFAP is so clearly pronounced that its immunochemical staining became a classical approach for visualization of normal astrocytes of the brain and of neoplastic cells of a glial origin (Fig. 1). In the mature brain, GFAP can be found in protoplasmic astrocytes within the gray matter, in fibrous astrocytes of the white matter, in radial (Bergmann's) glia of the cerebellum, and in subependymal astrocytes in the cerebellar ventricles. On the surface of the brain, GFAP-immunoreactive cells are present only as astrocytes forming the *glia limitans*. It is intriguing that astrocytes localized in various cerebral structures differ from each other in the ability to synthesize GFAP; astrocytes in the white matter express GFAP in greater amounts than those in the gray matter [1, 4].

As has been found, there are certain interspecies peculiarities of the synthesis of GFAP. Cerebral astrocytes in humans are capable of synthesizing GFAP by an order of magnitude more intensely than those in rodents. Beyond the bounds of the brain, GFAP is synthesized by astrocytes and Müller's cells of the retina participating in communication among neurons, ganglion cells, and endotheliocytes [17]. In the peripheral nervous system (PNS), mainly non-myelinated Schwann cells surrounding nerve fibers of the somatic nerves (sciatic nerve in particular), as well as autonomic nerve fibers in visceral organs, belong to the GFAP-positive cells [18-20]. The presence of such cells in the enteric nervous system (ENS) has also been described [21] (Fig. 2).

Over the past years, more and more information on the unusual localization of GFAP in non-nerve tissues became available; however, the amount of this protein in this case is rather scanty, as compared with that in astrocytes. "Soft" techniques for processing of the tissues and the use of high-affinity antibodies recognizing only specific epitopes of GFAP allowed researchers to identify this protein in epithelial cells of the crystalline lens, Leydig cells, Kupffer cells of the liver, cells of the pancreas, podocytes, mesangial cells, chondrocytes, and osteocytes [1, 13, 22]. Anti-GFAP antibodies clearly label fibroblasts and keratinocytes in rodents and humans [23]. GFAP was found in cells of the spinal cord during its regeneration [24]. Expression of GFAP is typical of the cells of some tumors of the salivary glands and pineal gland, papillary meningiomas, cartilage

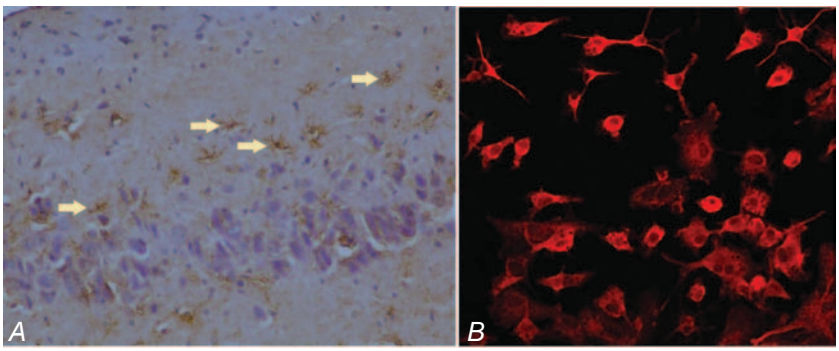

Fig. 1. Photomicrographs of immunohistochemically labeled astrocytes in the brain. A) Slice of the rat hippocampus; arrows indicate astrocytes that bound antibody against glial fibrillary acidic protein (GFAP) and were visualized using secondary antibody conjugated with peroxidase (magnification 100×). B) Confocal microscopy of astrocytes in primary hippocampal culture; red color (astrocytes that bound antibody against GFAP; secondary antibody, goat anti-rabbit IgG Fc, conjugated with Alexa Fluor 647; magnification 200×). Our own data unpublished earlier.

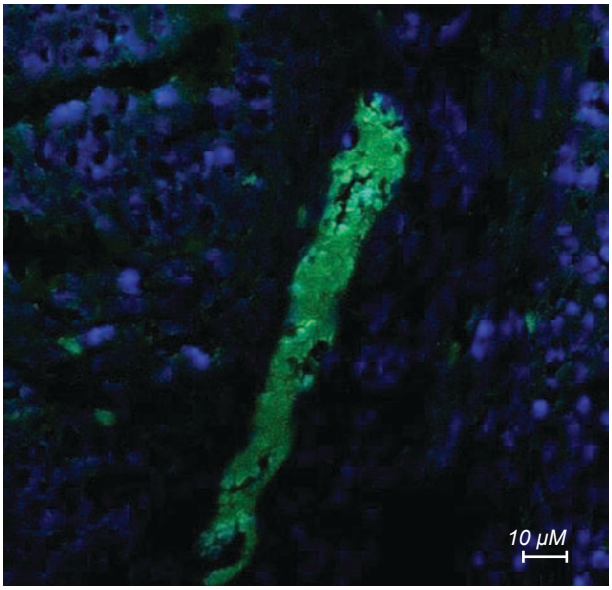

Fig. 2. Confocal photomicrograph of GFAP-positive cells belonging to enteric glia of a calf fetus. Green color) GFAP-positive cells, secondary antibody, goat anti-rabbit IgG, conjugated with FITC; blue color) cell nuclei stained with Hoechst 33342. Our own data unpublished earlier.

cells of the *epiglottis*, normal pituicytes, and cells of pituitary adenomas and malignant carcinomas of kidneys [1, 25, 26].

MOLECULAR BIOLOGY OF GFAP AND ITS ISOMORPHIC COMPOSITION

The human *GFAP* gene localized in chromosome 17q21.1-q25 (~ 10 kb DRA) was cloned for the first time in 1989 [27]. This gene is formed of eight introns and nine exons; among them there are four alternative exons and two transcribing mRNAs (~ 3 kb). Alternative splicing results in a rather significant polymorphism of GFAP. At present, 10 isoforms of GFAP have been isolated and described; different subtypes of this protein, GFAP-α, -δ(-ε), and -κ, were characterized, in particular, most completely [2, 28]. GFAP-α (isoform 1) consisting of 432 amino acid residues (a.r.) is the most widespread isoform in the brain and spinal cord. GFAP-δ-(-ε) (isoform 2) is formed of 431 a.r. and demonstrates an alternative sequence of residues in the C-terminal domain; this isoform is predominantly expressed by brainstem nerve cells of the subventricular cerebral zone. Indeed, because of the unique structure of the C-terminal sequence, the above-mentioned isoform interacts in a specific mode with presenilins-1 and -2. This phenomenon can play an important role in Notch signalling and determination of the future of the cells (obtaining of the GFAP-positive, i.e., astrocytic, phenotype or the neurogenic one) [29]. It seems possible that GFAP-δ-(-ε) is involved in the processes of migration and malignant transformation of cells. Increased expression of this isoform was demonstrated in cells of tumors of astrocytic origin. GFAP-κ (isoform 3; 328 a.r.) was isolated from the mouse cerebral cortex, striatum, and cerebellum. GFAP-β is formed of no less than 432 a.r.; this isoform prevails in non-myelinated Schwann cells of the PNS. Increases in the level of mRNA of GFAP-β and in the level of this protein *per se* result from injuries of the nerve fibers [30]. GFAP-γ (431 a.r.) and its mRNA were found in the CNS (*corpus callosum*) and also in non-nerve tissues, e.g., in the bone marrow and spleen. A primary sequence of GFAP-ξ consists of 438 a.r. or more [31].

There are additional four GFAP isoforms that come into existence due to a shift of the reading frame by one nucleotide (i.e., due to frame-shift mutation). They received the general name GFAP+1 (GFAPΔEx6, 347 a.r., GFAPΔ164, 366 a.r., GFAPΔ135, 374 a.r., and GFAPΔEx7, 418 a.r.) [6]. These isoforms were isolated, as a rule, from reactive astrocytes of different zones of the brain of patients suffering from Alzheimer's disease. It should be noted that three GFAP+1 splice forms were identified in pyramidal neurons of the hippocampus obtained from patients with Alzheimer's disease and Down syndrome, as well as from astrocytes just in the sites of focal lesions of the brain in patients suffering from epilepsy [33]. Unfortunately, the complicated control mechanisms underlying alternative GFAP splicing remain little studied. Questions of whether all products of alternative splicing are translated into the protein and what is the role of the ratio of different GFAP isoforms in regulation of the processes of assembling of IFs in astrocytes remain open.

More than 80 mutations of the *GFAP* gene have been described, the overwhelming majority of which was found in patients with Alexander disease [34]. Since most mutations fall on the encoding site of the *GFAP* gene, and only several ones develop in the promotor sequence, 95% of all mutations found in the above-mentioned disease are of the functional character. The mechanism of pathogenesis of this neurodegenerative disorder lies in the formation of accumulations of cytoskeletal structures (Rosenthal

fibrillae) inside astrocytes. These structures are constructed from a defective GFAP and the so-called heat-shock proteins, and they possess clearly pronounced cytotoxic properties. Alexander disease is a striking example of practically total loss of the function of GFAP, which is rather important for investigation of this pathology [35].

STRUCTURE AND PROPERTIES OF GFAP. PRINCIPLES OF FORMATION OF FILAMENTS

A product of translation of α-GFAP mRNA is the polypeptide with the molecular mass (Mm) ~ ~ 49,8 kDa, which is a monomer subunit of glial IFs. The main pool of GFAP is insoluble in water. This protein is characterized by a high ability for aggregation and polymerization and also by a sensitivity to neutral protease-induced proteolysis. In the molecule of GFAP, high-specific antigenic epitopes are present. GFAP is the amphiphilic protein that demonstrates the affinity for hydrophobic radicals [1, 2, 36]. The high evolutionary conservatism of the structure and homology of the amino acid composition of GFAP (demonstrated for different specimens of the animal kingdom [37]) explains the rather low level of its species-related specificity, which is confirmed by crossed immunoreactivity (Fig. 3).

Within the early postembryonal period, undifferentiated precursors of astrocytes express very limited amounts of GFAP. In such cells, IFs are formed of vimentin, and the latter is gradually replaced by GFAP in the course of ontogenesis [38]. The most intense synthesis of this protein is observed within the first months of the neonatal development; however, in mammals, the complex age-dependent remodeling of astrocytes is realized throughout their life. During ontogenesis, the intensity of GFAP expression gradually decreases [39]. There is, however, the second wave of expression of this protein typical mostly of patients with Alzheimer's disease; it is also observed under conditions of development of nonspecific (soft) gliosis related to brain aging [40]. In astrocytoma of IV degree, a dramatic drop in the level of GFAP is observed; this can be indicative of the loss of the ability of malignant cells to synthesize the IF protein during their dedifferentiation [41].

Activation of transcription of the *GFAP* gene is induced by numerous growth factors, such as ciliary (glial) neurotrophic factor (CNTF), fibroblast growth factor (FGF), transforming growth factor β (TGF-β), and interleukinin-6 [40]. Thyroid hormone and glucocorticoids also promote intensified transcription of the *GFAP* gene (probably, via activation of the ROCK pathway) [42]. Trophic factors of glial origin (glial neurotrophic factor, GDNF, neurturin, and artemin) provide activation of the autoregulatory cytoprotective mechanism targeted, in particular, on the maintenance of GFAP synthesis. These and other cellular regulators induce activation of astroglia either directly (via the STAT3 signalling in astrocytes) or indirectly (via the influences on microglial cells), endotheliocytes, and neurons [43, 44].

In the brain of different animals, integral regulation of expression of the *GFAP* gene is provided by neuroendocrine and inflammatory mediators [45]. It has been demonstrated that the neurogenic potential of astrocytes and the direction of differentiation of astrocytes are determined by several factors, in particular by transforming growth factor (TGF)-β1, peroxisome proliferator-activating receptor γ (PPARγ), and factor controlling the cellular cycle (TP53, or tumor protein). The effects of all these factors are associated with inflammatory processes; thus, inflammatory signal pathways can control differentiation of astrocytes and the profile

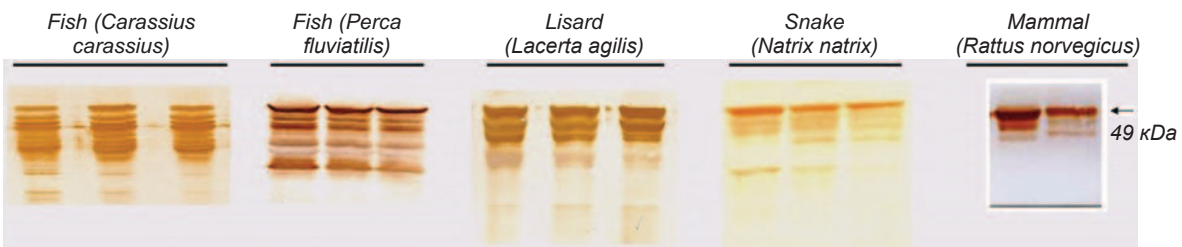

Fig. 3. Immunoblotting of GFAP in protein samples obtained from cerebral tissues of different vertebrates under conditions of the development of astrocytosis (our own data unpublished earlier).

of expression of the genes encoding specific proteins in the norm and under conditions of a reactive response of glial cells [46].

Similarly to all IF proteins, GFAP is constructed according to the unitary principle (Fig. 4). In a structure of the molecule of this protein, researchers distinguish a homologous central part (rod domain), which consists of nearly 310 a.r. forming a rather vast α-spiral region. This region contains tandem repetitions consisting of seven different a.r. (heptad repetitions). The central GFAP domain mediates interaction between two monomers with the formation of a spiraled dimer structure. The rod domain is the most conservative in all types of IFs, while terminal domains of the molecule are hypervariable parts of the polypeptide chain. In most IFs, the globular N and C domains form "hands" on the surface of filament, which mediate interaction of this filament with other ones and also with other intracellular structures. In contrast to "hands" of the neurofilaments, the corresponding components of the surface of astrocytic filaments are not extended; they are organized more densely and spread in the cytoplasm of astrocytes as compact bunches [47, 48].

As was demonstrated, there is a certain hierarchy of the structure of the filament: monomer→dimer→tetramer (Fig. 5). Just tetramers form the filament *per se*; the latter is a symmetric nonpolar structure. Homomeric polymers of GFAP are formed at the expense of interaction of the N-terminal head domains. Under *in vivo* conditions, GFAP exists as a heteropolymer; it is polymerized together with vimentin and/or nestin [13, 49].

Assemblage of the astrocytic IFs is controlled by amino acid replacements induced by gene mutations, post-translational modifications, competitive interactions of different GFAP isoforms, and interactions with other proteins (S100, annexin, vimentin, α-crystalline, etc.). Intermediate filaments are very stable structures, as compared to microtubules and microfilaments; reorganization of IFs in the cell is realized rather rarely [50, 51].

As the published data and our own findings show, partly degraded GFAP polypeptides, which constitute the native filaments, are intensely extracted and enter into a fraction of the cerebral proteins insoluble in water under conditions of action of different unfavorable factors, in particular during the development of neuropathies (Fig. 3) [52-54]. Taking into the account these observations, it has been hypothesized that limited proteolysis

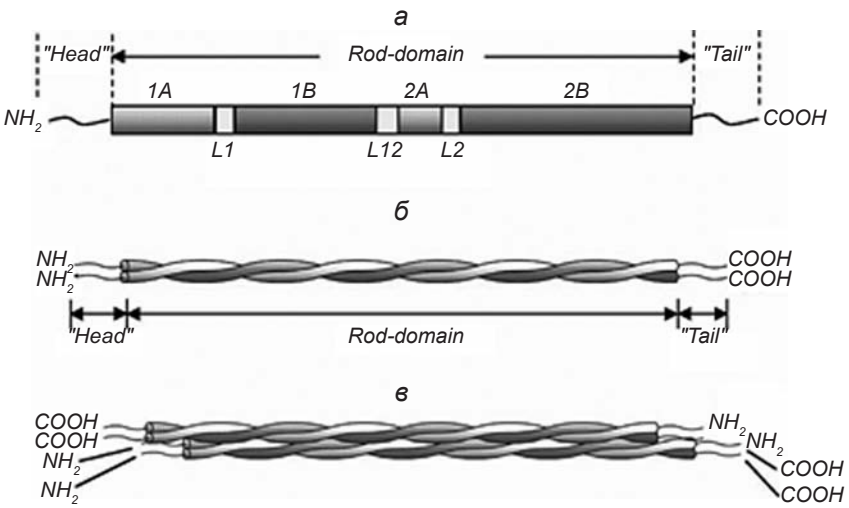

Fig. 4. Scheme of the GFAP structure. A) Monomer, B) parallel dimer, and C) antiparallel tetramer. 1A, 1B, 2A, and 2B) Parts of the super-spiraled α-spiral split by non-spiraled spacer chains (L1, L12, and L2). Spiraled parts of each chain contain 5 (1A), 14-15 (1B), 2-3 (2A), and 17-18 (2B) blocks of amino acid residuals; the head is the hypervariable N-terminal domain, the rod-domain is the constant domain, and the tail is the hypervariable C-terminal domain [47, 48].

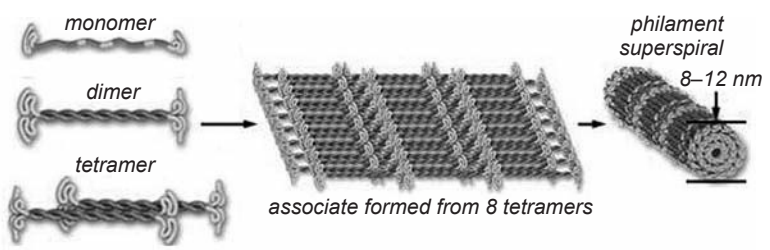

Fig. 5. Hierarchic principle of the structure of an intermediate filament [50, 51].

of proteins (which form the filament) can be a single pathway of reorganization of the fibrillar astrocytic apparatus that demonstrates high stability in the norm. As has been proposed, the level of fragmentation of GFAP should be used as an index of the development of reactive astrocytosis, which is supplemented by the data of quantitative analysis of this protein [55, 56]. The amount of GFAP breakdown products in biological fluids of the organism is used as an index of severity of the trauma of the spinal cord and damage to the CNS [57, 58].

A unique peculiarity of GFAP, which distinguishes this protein from other homologous proteins, is the presence of a small pool of water-soluble polypeptides in its composition. A soluble GFAP fraction was obtained, for the first time, from the nerve tissues of humans and animals *post mortem*. The origin of this fraction can be related to activation of calcium-dependent neutral proteinase [1, 59]. In recent years, it was, however, proved that non-polymerized soluble GFAP polypeptides can exist in the brain *in vivo*; their appearance in appreciable amounts is observed under the action associated with the calcium-mediated mechanisms of phosphorylation and proteolysis [60]. Despite the fact that there is significant progress in the studies of GFAP, the question of the functional importance of soluble polypeptides in the composition of the integral pool of this protein remains open.

METABOLISM OF GFAP: POST-TRANSLATIONAL MODIFICATIONS AND DEGRADATION

Among the main post-translational modifications of GFAP, there are phosphorylation, citrullination, glycosylation, and acetylation [13, 61]. Phosphorylation of GFAP is realized mostly by protein kinase C (PKC), protein kinase A (PKA), and calmodulin-dependent protein kinase II (CAMPKII). These enzymes are key agents involved in the processes of intracellular signalling. Six amino acid residues, five of which are localized in the N-terminal domain of the primary GFAP sequence, are subjected to phosphorylation. The residues and enzymes providing their phosphorylation are the following: Thr-7 (PKA), Ser-8 (PKA, PKC, cdk2), Ser-13 (CAMPKII, PKA, PKC), Ser-17 (CAMPKII), Ser-38 (CAMPKII, PKA, PKC), and Ser-289 (CAMPKII). Since many sites of GFAP phosphorylation are localized in the N-terminal part, this modification of the protein inhibits the process of assemblage of the filament. There is evidence that phosphorylation of GFAP provides regulation of cell-to-cell interaction between neurons and astrocytes [62, 63]. Snider and Omary [64] emphasized the importance of aberrant phosphorylation of GFAP for pathophysiological processes in the nerve tissue realized with the involvement of astrocytes. In particular, the level of GFAP phosphorylation, which is intensified during hypoxia/ischemia of the brain, determines the ability of astrocytes to remodeling. Therefore, this factor plays an important role in the plasticity of the nervous system under pathophysiological conditions and during reparative processes [65].

Recently, it has been found that endogenous citrullination of GFAP can be realized at the expense of deamination of arginine residues in the positions of Arg-30, -36, -270, -406, and -416. The appearance of citrulline residues in the composition of GFAP lends to the product properties of an autoantigen against this protein during the development of some neurological disorders; however, the physiological importance of citrullination of the protein in the norm remains unknown [66]. Polypeptides of GFAP acetylated by residues of lysine in positions of Lys-89, -153, -189, -218, -259, and -331 were found in the spinal cord of patients with amyotrophic lateral sclerosis [67].

Some proteolytic enzymes are responsible for splitting of GFAP; as a result of such splitting, more or less degraded polypeptides appear. As was proved, the leading role in degradation of GFAP is played by calcium-dependent protein kinases (calpains), which specifically hydrolyze peptide bonds in both C- and N-terminal sites of the protein. A result of calpain-mediated proteolysis of GFAP is the appearance of some degraded polypeptides with the molecular mass from 38 to 44 kDa. Such polypeptides were found under *in vitro* conditions in cultured astrocytes, as well as in cerebral tissues and cerebrospinal fluid of mammals in different natural and experimentally induced pathologies (in the spinal structures of patients with amyotrophic lateral sclerosis and in the spinal cord of animals after traumatization) [1, 68]. A specific site of splitting of GFAP by calpains is the position of Thr-383-Phe-384, although some researchers cannot rule out the possibility that proteolysis of this protein can be realized in other sites of its primary sequence. The main product of degradation of GFAP by calpains is the fragment with the

Mm ~38 kDa, which consists of an intact rod domain and shortened N- and C-terminal domains. Taking into account the leading role of the latter in the processes of assemblage and organization of the fibrils, it is hypothesized that such a structure prevents the involvement of this degraded polypeptide in the formation of the filament [69].

Caspases-3 and -9, which provide degradation of the protein under conditions of a pro-apoptotic state of the cell, also play a significant role in proteolysis of GFAP. A GFAP fragment with the Mm ~20 kDa and a shortened N terminal isolated from the brain of patients with Alzheimer's disease was identified as a product of incomplete splitting of GFAP by caspases [54]. Recently, it has been demonstrated that there is a possibility that GFAP can be split by the cysteine proteinase caspase-6, which results in the formation of two fragments with the Mm 24 and 26 kDa. In this case, the C-terminal GFAP fragment (C-GFAP, 24 kDa) was not capable of polymerizing, while the longer chain (N-GFAP) could form filament-like structures. The latter, however, did not demonstrate the ability to form more complex aggregates [70].

FUNCTIONS OF GFAP

Studies on transgenic animals, in particular on *GFAP* gene-knockout ones, and the use of antisense RNAs open up broad prospects for investigation of the functions of this cytoskeletal protein in the CNS [1, 2, 12, 13].

Functioning of GFAP in the Composition of the Astrocytic Cytoskeleton. According to the classical concept, filament structures formed of GFAP play a key role in the maintenance of morphology of astrocytes and stabilization of their numerous processes, as well as in the processes related to cell migration. However, the data that GFAP can possess kinase-binding sites are indicative of the possibility for a significant involvement of this protein in the processes of cellular signalling. This is also confirmed by the finding that astroglial IFs are capable of forming a dynamic continuum with other cytoskeletal structures and also (via integrin receptors) with the components of the extracellular matrix [71].

Studies of the role of GFAP in physiological processes realized with the participation of astrocytes are being continued; however the obtained data and their interpretation remain rather ambiguous. In one of the first such studies [72], it was demonstrated that the complete absence of GFAP, beginning from the embryonal period, practically did not influence the development and morphology of the CNS in mice. A possible explanation of this fact is the following. The absence of GFAP can partly be compensated by expression of a homologous protein, vimentin. However, the data obtained by other authors did not confirm this supposition but are indicative of the existence of considerable long-term negative effects in animals with GFAP dysfunction. Experiments carried out on mutant mice showed that GFAP plays an important role in the formation of architecture of the white matter and in the maintenance of integrity of the BBB. In GFAP-deficient mice, it has been demonstrated that hydrocephaly combined with loss of the white matter in the brain [73] can develop in these animals. In GFAP(-/-) mice with experimental autoimmune encephalomyelitis (model of multiple sclerosis), aberrantly organized vimentin-containing IFs were formed in astrocytes under conditions of the absence of GFAP; in such animals, the level of demyelination was higher than that in wild-type mice [74]. The GFAP deficiency was reflected in the transport of some important metabolites to astrocytes [75]. Studies of Tardy et al. [76] proved the key role of GFAP and the process of its phosphorylation in the mitosis of astrocytes. Of special interest are the data obtained by several independent research groups. The importance of GFAP for the processes of astrocyte-to-neuron communication has been demonstrated. In GFAP-knockout mice, researchers observed disturbances in the formation of long-term types of synaptic plasticity (depression and potentiation) in neuronal structures of the hippocampus and cerebellum [77]. In these mice, astrocytes were not able to form cellular processes under conditions of co-culturing with neurons [78]. Altogether, these data allow one to state that the normal organization of GFAP-containing filaments of the astrocytic cytoskeleton determines the adequacy of cell-to-cell interaction in the CNS and is responsible for the processes of synaptic transmission modulated by astrocytes.

Role of GFAP in Reactive Astrocytosis. Damage to the nerve tissue induces intense proliferation and hypertrophy of astrocytes; these shifts are accompanied by accelerated synthesis of GFAP and fibrillogenesis. This phenomenon (known as *astrocytosis*) is the main link in the development of reactive gliosis as the total glial response of the CNS to damage [79]. GFAP is directly involved in

the reactivation of astrocytes and the maintenance of morphology of their processes. The localization and coverage of glial reactivation under conditions of pathology of the CNS depend on the level of injury and can exert different effects on the neuronal environment. It is well known that, on the one hand, gliosis fulfills certain neuroprotective function due to the presence of some neurotropic substances synthesized and secreted by reactive cells. On the other hand, excessive gliosis and formation of a dense glial cicatrix can exert significant negative effects on structural and functional recovery of the nerve tissue due, first of all, to overexpression of proinflammatory factors and the development of ischemia [11, 80]. Recently, Pekny et al. [80] reported the leading role of GFAP in the formation of the glial cicatrix. Ghirnikar et al. [81] showed that, in double mutant mice incapable of synthesizing both GFAP and vimentin, the glial cicatrix could be formed after damage to cerebral tissues, but the density of such cicatrix was insufficient for the maintenance of the BBB integrity. These data indicate that the adequate structure of astrocytic IFs is an essential prerequisite for the development of the corresponding astrocytic response after damage to the CNS. It has also been demonstrated that injury of a peripheral nerve in GFAP(-/-) mice resulted in the disturbance of differentiation of Schwann cells and also in a delay of the regenerative processes [82]. In experiments carried out on pheochromocytoma cells (cell strain PC-12) transfected with *GFAP* gene, it was proved that the presence of this protein is very important for survival of the cells in stress: PC-12 cells with overexpression of GFAP showed clearly increased resistance to thermal shock [83]. Anti-apoptotic effects supposedly observed with intensification of the GFAP synthesis need further studies, since the regulation of expression of this protein determines, to a significant extent, the neuroprotective properties of astrocytes after damage to the nerve tissue and development of neurodegenerative disorders.

The astroglial reaction to injury, the rate of formation of such a response, and evolutionary conservatism of reactive astrogliosis are indicative of the importance of the functioning of reactive astroglia in the CNS. In addition to fibrillogenesis and re-expression of vimentin, gliosis is frequently accompanied by reconstruction of the filament apparatus of astrocytes at the expense of activation of a calcium-dependent system of proteinases [84]. Therefore, proteolysis is one of the mechanisms underlying rearrangement of IFs during the astrocytic response. Another pathway necessary for the control of the state of the cytoskeleton is RhoA-mediated signalling between cellular membranes and proteins of the cytoskeleton [85].

Functions of GFAP in Non-Astrocyte Cell Units. Localization of GFAP among the cells that form a barrier between the organism and the environment is indicative of the possibility that this protein can fulfill fundamentally other functions differing from those in the nerve tissue. In particular, the involvement of GFAP in the formation and maintenance of mechanical and immunological barriers needs adequate examination.

The role of GFAP in enteric glia has been studied in detail. GFAP-immunopositive cells of enteric glia, which are localized in submucous and muscular layers of the intestine, fulfill a neuromodulatory function; this protein is responsible for the maintenance of functioning of the neurons and their communication with epithelial cells and participates in the control of intestinal peristalsis and myxopoiesis [86]. The intensity of GFAP synthesis by enteric glial cells is influenced by proinflammatory regulators (interleukin-6, bacterial endotoxin LPS, etc.). Intensification of synthesis of GFAP by enteric glial cells in response to the development of ulcerative diseases of the intestine, enterocolitis, and Crohn's disease can serve as a sensitive diagnostic criterion of the time course of these pathologies. Among enteric gliocytes, GFAP is involved in reparative processes related to damage to the intestine [87]. It has been found that the cells of enteric glia in mice can play the role of precursors of neurons in the case of injury of the intestine [88]. Functional homology of GFAP-synthesizing cells in the CNS and ENS is confirmed by data on the rise in the amount of enteric GFAP and in the level of its phosphorylation in the intestine of patients with Parkinson's disease [21].

GFAP AS A MARKER OF DAMAGE TO NERVE TISSUE

Changes in the amount of GFAP resulting from injury of the CNS determine the importance of results of its quantitative analysis for estimation of the level of damage to nerve tissue. Based on identification of this protein in biological liquids, it can be concluded that some neurodegenerative diseases are accompanied by disorders of the BBB integrity [6, 11]. In USA, quantitative estimation of

GFAP has been included in the list of neurotoxicity risk assessment during preclinical testing of drugs [89].

GFAP under Conditions of Neurodegenerative States. Reactivation and overexpression of GFAP of astrocytic IFs result from a wide range of factors and pathological states. Among them, there are some viral infections, encephalopathies related to prion infection, diseases accompanied by inflammation and demyelination (in particular, allergic encephalomyelitis), acute trauma of the brain, ischemia/hypoxia, cryogenic damage to the brain, and influence of chemical toxicants. The latter include ions of aluminum, lead, and mercury, acrylamide, ethanol, β,β'-iminodipropionitrile, di- and trichloroethane, xylene, triethyltin, colchicine, kainates, 6-hydroxydopamine, etc. Intensified expression of GFAP can be caused by the action of ionizing radiation, brain irradiation with extra high-frequency electromagnetic waves, metabolic disorders (*diabetes mellitus*, hyperthyroidism, hyperphenylalaninemia, and aceruloplasminemia), etc. Intensification of proliferation of astrocytes and fibrogenesis are observed in different cerebral zones during the development of some neurodegenerative diseases, namely amyotrophic lateral sclerosis, Gerstmann–Straussler syndrome, and Huntington's, Wilson's, Pick's, and Parkinson's diseases [1, 11, 90-98]. Astrocytic gliosis is a typical neuropathological sign of Alzheimer's disease. Numerous experimental data indicated that reactive astrocytes in the brain of patients with Alzheimer's disease are associated with neurites and amyloid plaques. In patients with manifestations of this disease, GFAP is frequently subjected to citrullination and oxidation. The role of reactive glia in the formation of β-amyloid aggregates remains little known at present [97-99].

Studies that dealt with the ability of astrocytes to synthesize GFAP provide certain data on the action of the states related to suppression of metabolic activity of these cells. Inhibition of GFAP synthesis as a manifestation of suppression of metabolic activity of astrocytes is observed in acute viral infections and chronic neurodegeneration. It has been demonstrated that HIV-1 infection causes a decrease in the amount of GFAP astrocytic IFs; in this case, a viral capsid glycoprotein, gp120, directly inhibits phosphorylation of GFAP [100]. Chronic infections caused by *varicella zoster* virus and suid herpesvirus (exciter of Aujeszky's disease, the so-called pseudorabies accompanied by the development of encephalomyelitis) inhibit synthesis of GFAP [101, 102]. Abnormally low expression of GFAP was found in samples of cerebral tissues obtained *post mortem* from patients with Down syndrome, schizophrenia, and bipolar affective disorder [103].

Identification of GFAP in different biological fluids (first of all, in the blood and cerebrospinal fluid, CSF) is of potentially high diagnostic importance. A great amount of information on a significant rise in the circulating and/or liquor pool of GFAP in patients suffering from *neuromyelitis optica* (Devic's disease), multiple sclerosis, ischemic stroke, subarachnoid hemorrhages, vasculitis of cerebral vessels, Alzheimer's disease, hydrocephaly, and also in persons suffering from narcolepsy has been gathered [13]. The appearance of GFAP in the amniotic fluid was described in experimental models (*myelomeningocele* and defects in the formation of the neural tube) [104]. Damage of the integrity of the barrier structures is the main reason for the release of GFAP and its entry into the blood and CSF during traumas of the brain and spinal cord. Increase in the concentration of GFAP in the blood serum resulting from secondary brain injury caused by subarachnoid hemorrhage and enhancement of the intracranial pressure has been described [105]. It is intriguing that the amount of GFAP in the blood serum and CSF in patients suffering from schizophrenia and in healthy humans was found to be the same; the data on the presence of GFAP in the above-mentioned fluids in patients with Parkinson's disease are contradictory [13, 106]. Evaluation of the concentration of circulating GFAP is used as a diagnostic marker for estimation of the level of development of gliomas [107]. Questions related to the specificity of GFAP isoform composition found in this case in the blood and CSF and also to the possibility of post-translational modifications of this protein, including proteolytic degradation, remain open.

In some studies, the authors proposed that evaluation of the titers of autoantibodies against GFAP should be used as a biomarker for the level of severity of different neurodegenerative states and also for the effects of neurotoxic substances. The synthesis of anti-GFAP-IgG is realized during the first four days after trauma of the brain [108]. It has been found that the appearance of autoantibodies against GFAP can be a manifestation of the development of autoimmune astrocytopathies and can be observed during the development of gliomas

[109]. In addition to obvious clinical/diagnostic aspects, elucidation of the importance of post-translational modifications of GFAP (especially of its citrullinated form) for recognition of this protein by cells of the immune system and peculiarities of clearance of such autoantibodies and of their role in the pathogenesis of neuropathies is very urgent [110].

GFAP under Conditions of Alcohol Encephalopathy and Thiamine Deficiency. Encephalopathy induced by chronic alcohol intoxication is an exclusively widespread neurodegenerative state (ethyl alcohol is the most consumed neurotoxin in the whole world) [111]. It is well known that astrocytes play the main role in the processes of detoxication of both ethanol *per se* and products of its metabolism in the brain. The effects of ethanol on the state of astrocytes have been studied in detail; the obtained data are, however, rather contradictory [112]. This is related, first of all, to the fact that the pattern and direction of changes in the amount of GFAP depends on the dose and duration of consumption of this toxicant. It has been proved that relatively short-lasting consumption of ethanol in the experiment (10% ethanol solution, 4 weeks) leads to activation of astrocytes, which results in an increase in the number of GFAP-positive cells in brain slices obtained from experimental animals [113]. More prolonged exposure of animals to the action of ethanol causes destructive modifications in astrocytes. Such alterations are accompanied by degradation of proteins of the cytoskeleton and irreversible abnormal changes of the morphology of these cells (even after long-lasting rehabilitation, the norm was not reached) [114]. Examination of the effects of alcohol consumption by pregnant female rats within the lactation period on the state of astroglia in offspring showed that the levels of GFAP in the hippocampus and cerebellar cortex of such young rats within the early postnatal period decreased significantly [115]. The effect of ethanol during embryogenesis induces a drop in the amount of both GFAP mRNA and this protein *per se*. This is realized not only in the brain of newborn rats but also in cultured glial cells obtained from the cerebral tissue (primary culture). It was found that ethanol is capable of directly disturbing the processes of regulation of expression of the *GFAP* gene because of hypermethylation of the corresponding DNA site [116]. Expression of GFAP is suppressed in the brain of rat fetuses by the action of ethanol, also at the expense of suppression of differentiation of the cells belonging to radial glia (precursors of astrocytes). Based on the available data, we hypothesize that the early alcoholization-caused modifications in the profile of specific proteins in astrocytes induse significant long-term effects, which are negatively reflected in the proliferation and migration of neuronal precursors, morphogenesis of neurons, processes of axonal sprouting, synthesis of trophic factors, and myelination [117]. Thus, the quantitative deficiency of astrocytes and destruction of GFAP-containing structures (phenomena observed under chronic action of ethanol, especially within the prenatal period) are key links in the pathogenesis of alcohol-induced encephalopathy and neuropsychological disorders associated with the latter [118-120].

It is known that one of the manifestations of alcohol-induced encephalopathy is thiamine (vitamin B_1) deficiency. Such a deficiency is the main pathogenetic factor of the development of the Wernicke–Korsakoff syndrome in humans suffering from chronic alcoholism [121, 122]. Long-lasting consumption of ethanol leads to inhibition of thiamine pyrophosphokinase, an enzyme responsible for phosphorylation of the intracellular pool of thiamine with the formation of its co-enzyme form, TDP. The slowdown of formation of TDP in chronic alcoholism causes inhibition of the activity of mitochondrial dehydrogenases and transketolases in the brain of patients with Wernicke–Korsakoff syndrome. This, in turn, results in dysfunction of the mitochondria in nerve cells, as well as in the development of oxidative stress [123]. It should be taken into account that thiamine deficiency accompanies most neurodegenerative pathologies not related to alcohol consumption and can serve as a factor of initiation of development of some such pathologies [124]. It is well known that astrocytes (cells of the nerve tissue) possess the maximum sensitivity to thiamine deficiency; dysfunction of these cells under conditions of B_1 deficit plays the leading role in the pathophysiological processes accompanying the development of avitaminosis [125]. Within the symptomatic stages of thiamine deficiency (9 to 14 days), the level of GFAP remains unchanged [126] or tends to increase [127]. Overexpression of GFAP was observed against the background of a significant decrease in the number of neurons, which was estimated by the neuronal marker NeuN. At the same time, in the case where vitamin B_1 deficiency was dramatic (as in the case of action of ethanol intoxication), the number of

GFAP-positive cells decreases significantly [128]. To generalize the above-mentioned literature data, we conclude that the initial stages of alcohol encephalopathy and thiamine deficiency are characterized by active proliferation of astrocytes. Such proliferation can be considered a compensatory reaction of the CNS to the developing metabolic disorders. Serious disorders of metabolic processes, which are accompanied by death of cerebral cells, result in suppression of the function of glia and irreversible alterations of the cytoskeletal structures in gliocytes.

GFAP and Aging of the Brain. It is believed that aging is a risk factor with respect to many pathological disturbances, including neurodegenerative disorders [129]. As to the involvement of GFAP in age-related processes, the following paradigm dominated for a long time: Aging of the nerve tissue is accompanied by accumulation of GFAP in astrocytes. Increase in the content of GFAP, as a manifestation of brain aging in humans, was demonstrated for the first time in the hippocampal structures and then in the cerebral cortex [130]. Accumulation of GFAP was not considered to be related to inhibition of catabolism of this protein. Results of immunoblotting indicated that a rise in the level of intact IF subunits appears concurrently with the enhancement of the amount of products of GFAP degradation in all samples of the senescent brain. Moreover, it has been demonstrated that the soluble pool of GFAP increases with age [131]. Significant differences in the expression of GFAP in mice clones characterized by various sensitivities to senescence were shown using immunohistochemistry techniques, immunoblotting, and the polymerase chain reaction. The level of GFAP in the brain was significantly higher in the senescence-accelerated animal clone than that in the senescence-resistant clone, which is indicative of the important role of the functional state of glial IFs in age-related processes [132].

Despite the widespread viewpoint with respect to the rise in the expression of GFAP and reactivation of astrocytes during aging [133], more and more convincing data indicate that the increase in the content of GFAP under conditions of normal aging is not related to neurodegeneration; it is an independent process corresponding to the adaptive response of astrocytes to age-related modifications in the nerve tissue [134, 135]. In particular, researchers observed increases in the number of GFAP-positive cells in the neocortex and subcortical white matter of senescent primates, but astrocytes in this case demonstrated no signs of hypertrophy [131]. In the brain of healthy elderly persons, the level of gliosis is relatively moderate [136]. There are certain differences in the profile of GFAP expression in different cerebral regions. In particular, there were no significant age-related changes in this index in the cochlear nuclei of the human brain in 20- to 30-year-old and 90-year-old examined subjects [137]. Of special interest are the data on modulation of GFAP expression in some parts of the hippocampus during ontogenesis, since this brain structure is responsible for commutation of signals incoming from most cerebral regions and provides processes related to learning and memory. It has been demonstrated that hippocampal astrocytes are extremely rapidly transformed in adult forms during the first month of postnatal development. During the next two months, hippocampal astrocytes are slowly aggregated in stable ensembles; however, the ability of these cells for rapid reactivation and morphological rearrangements is obvious even in elderly persons [138]. Increases in the number of GFAP-positive cells in the rat brain (within a time interval from 12 to 24 months of life) were found in the frontal cortex and in the hippocampal *CA1* area. With aging, the number of astrocytes increases more intensely in the hippocampus than in the neocortex; however, hypertrophy of astrocytes is more expressed just in the latter structure [139]. In the brain of old mice (59 weeks old), a maximum rise in the level of GFAP was found in the hippocampal *CA1* area [140].The immunoreactivity of GFAP in all layers of this area of the hippocampus in aged dogs was significantly higher than that in adult younger animals [141]. Taking into account the leading role of the hippocampus in the realization of the processes of learning and memory, it was hypothesized that glial activation and intensification of GFAP expression can be significant pathogenetic factors that cause the impairment of neuronal plasticity in some cerebral regions with further development of cognitive deficiency [142].

With aging of the brain, a number of factors can modulate synthetic activity of astrocytes, including the capability of these cells to express GFAP. In particular, an insufficient amount of docosahexaenoic acid (DHA) is reflected in the ability of astrocytes for reactivation. Under conditions of DHA insufficiency, the reactivity of astrocytes and the intensity of metabolic processes in the brain of aged rats were two times lower than those in young animals [143]. It was found that testosterone injected into the rats

under study prevents age-related enhancement of the level of GFAP in the cerebellum, i.e., in the cerebral structure very sensitive to the action of steroid hormones [144]. The authors of the cited paper study hypothesized that the decrease in the level of circulating testosterone can be the age-related reason for the rise in the amount of GFAP in the above-mentioned brain structure. At the same time, Anderson et al. [145] demonstrated that the gradual increase in the amount of GFAP mRNA in different regions of the brain is not related to sex differentiation.

When summarizing the data presented in our review, it should be emphasized that IFs of the cytoskeleton of astrocytes are universal intracellular structures; the high evolutionary conservatism of GFAP is indicative of the homology of the functions fulfilled by this protein in astrocytes and some other types of cells in different phylogenetic groups of organisms. In cytoskeletal structures, GFAP is involved in the realization of a number of the processes; namely, it provides adequate functioning of astrocytes and regulates glia-to-neuron interactions in the norm and during the development of reactive gliosis induced by different neurotoxic/traumatic factors and neuropathological states. Taking into account that the functioning of glial IFs is involved in a wide range of processes, GFAP can be a suitable target for modulation of the functions of these cells. The unique peculiarities of functioning of GFAP as the structural/integrative component of the intracellular space, as well as the component of the cellular signal systems, need further interdisciplinary investigations.

This paper is a literature review; it was not associated with any experiments on animals or tests involving human objects. Considering this, conformation of the study to the existing ethical standards for experimental studies is not required.

The authors, A. A. Tykhomyrov, A. S. Pavlova, and V. S. Nedzvetsky, confirm that they have no conflict of interest pertinent to commercial or financial relations and relations with organizations or persons somehow or other related to the study, as well as to interrelations within the authors' group.

REFERENCES

1. L. F. Eng, R. S. Ghirnikar, and Y. L. Lee, "Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000)," *Neurochem. Res.*, **25**, Nos. 9/10, 1439-1451 (2000).
2. Z. Yang and K. K. Wang, "Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker," *Trends Neurosci.*, **38**, No. 6, 364-374 (2015).
3. L. F. Eng, J. J. Vanderhaeghen, A. Bignami, and B. Gerstl, "An acidic protein isolated from fibrous astrocytes," *Brain Res.*, **28**, No. 2, 351-354 (1971).
4. C. T. Uyeda, L. F. Eng, and A. Bignami, "Immunological study of the glial fibrillary acidic protein," *Brain Res.*, **37**, No. 1, 81-89 (1972).
5. A. Bignami, L. F. Eng, D. Dahl, and C. T. Uyeda, "Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence," *Brain Res.*, **43**, No. 2, 429-435 (1972).
6. J. Middeldorp and E. M. Hol, "GFAP in health and disease," *Prog. Neurobiol.*, **93**, No. 3, 421-443 (2011).
7. H. Deka, R. Sarmah, A. Sharma, and S. Biswas, "Modelling and characterization of glial fibrillary acidic protein," *Bioinformation*, **11**, No. 8, 393-400 (2015).
8. S. A. Lewis and N. J. Cowan, "Temporal expression of mouse glial fibrillary acidic protein mRNA studied by a rapid *in situ* hybridization procedure," *J. Neurochem.*, **45**, 913-919 (1985).
9. M. V. Sofroniew and H. V. Vinters, "Astrocytes: biology and pathology," *Acta Neuropathol.*, **119**, No. 1, 7-35 (2010).
10. L. Ben Haim, M. A. Carrillo-de Sauvage, K. Ceyzériat, and C. Escartin, "Elusive roles for reactive astrocytes in neurodegenerative diseases," *Front. Cell Neurosci.*, **9**, 278 (2015).
11. L. F. Eng and R. S. Ghirnikar, "GFAP and astrogliosis," *Brain Pathol.*, **4**, No. 3, 229-237 (1994).
12. E. M. Hol and M. Pekny, "Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system," *Curr. Opin. Cell Biol.*, **32**, 121-130 (2015).
13. A. Petzold, "Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease," *Brain Res.*, **1600**, 17-31 (2015).
14. C. M. Jacque, C. Vinner, M. Kujas, et al., "Determination of glial fibrillary acidic protein (GFAP) in human brain tumors," *J. Neurol. Sci.*, **35**, No. 1, 147-155 (1978).
15. L. Schiff, N. Hadker, S. Weiser, and C. Rausch, "A literature review of the feasibility of glial fibrillary acidic protein as a biomarker for stroke and traumatic brain injury," *Mol. Diagn. Ther.*, **16**, No. 2, 79-92 (2012).
16. L. F. Eng, B. Gerstl, and J. J. Vanderhaeghen, "A study of proteins in old multiple sclerosis plaques," *Trans. Am. Soc. Neurochem.*, **1**, 42 (1970).
17. K. A. Wunderlich, N. Tanimoto, A. Grosche, et al., "Retinal functional alterations in mice lacking intermediate filament proteins glial fibrillary acidic protein and vimentin," *FASEB J.*, **29**, No. 12, 4815-4828 (2015).
18. K. R. Jessen, R. Thorpe, and R. Mirsky, "Molecular identity, distribution and heterogeneity of glial fibrillary acidic protein: an immunoblotting and immunohistochemical study of Schwann cells, satellite cells, enteric glia and astrocytes," *J. Neurocytol.*, **13**,

No. 2, 187-200 (1984).
19. D. Dahl, N. H. Chi, L. E. Miles, et al., "Glial fibrillary acidic (GFA) protein in Schwann cells: fact or artifact?" *J. Histochem. Cytochem.*, **30**, No. 9, 912-918 (1982).
20. G. B. Suarez-Mier and M. S. Buckwalter, "Glial fibrillary acidic protein-expressing glia in the mouse lung," *ASN Neuro*, **7**, No. 5, pii: 1759091415601636 (2015).
21. T. Clairembault, W. Kamphuis, L. Leclair-Visonneau, et al., "Enteric GFAP expression and phosphorylation in Parkinson's disease," *J. Neurochem.*, **130**, No. 6, 805-815 (2014).
22. S. Hassan, S. Syed, and S. I. Kehar, "Glial fibrillary acidic protein (GFAP) as a mesenchymal marker of early hepatic stellate cells activation in liver fibrosis in chronic hepatitis C infection," *Pak. J. Med. Sci.*, **30**, No. 5, 1027-1032 (2014).
23. L. Danielyan, S. Zellmer, S. Sickinger, et al., "Keratinocytes as depository of ammonium-inducible glutamine synthetase: age- and anatomy-dependent distribution in human and rat skin," *PLoS One*, **4**, No. 2, e4416 (2009).
24. M. Murray, S. D. Wang, M. E. Goldberger, and P. Levitt, "Modification of astrocytes in the spinal cord following dorsal root or peripheral nerve lesions," *Exp. Neurol.*, **110**, No. 3, 248-257 (1990).
25. S. S. Shah, V. S. Chandan, D. C. Wilbur, and K. K. Khurana, "Glial fibrillary acidic protein and CD57 immunolocalization in cell block preparations is a useful adjunct in the diagnosis of pleomorphic adenoma," *Arch. Pathol. Lab. Med.*, **131**, No. 9, 1373-1377 (2007).
26. P. Redecker and J. Fechner, "Immunohistochemical study of cells positive for glial fibrillary acidic protein (GFAP) in the human pituitary gland, with special reference to folliculo-stellate cells," *Histochemistry*, **91**, No. 3, 227-234 (1989).
27. S. A. Reeves, L. J. Helman, A. Allison, and M. A. Israel, "Molecular cloning and primary structure of human glial fibrillary acidic protein," *Proc. Natl. Acad. Sci. USA*, **86**, No. 13, 5178-5182 (1989).
28. D. F. Condorelli, V. G. Nicoletti, V. Barresi, et al., "Structural features of the rat GFAP gene and identification of a novel alternative transcript," *J. Neurosci. Res.*, **56**, No. 3, 219-228 (1999).
29. R. Thomsen, T. F. Daugaard, I. E. Holm, and A. L. Nielsen, "Alternative mRNA splicing from the glial fibrillary acidic protein (GFAP) gene generates isoforms with distinct subcellular mRNA localization patterns in astrocytes," *PLoS One*, **8**, No. 8, e72110 (2013).
30. D. F. Condorelli, V. G. Nicoletti, P. Dell'Albani, et al., "GFAPbeta mRNA expression in the normal rat brain and after neuronal injury," *Neurochem. Res.*, **24**, No. 5, 709-714 (1999).
31. J. Blechingberg, I. E. Holm, K. B. Nielsen, et al., "Identification and characterization of GFAPkappa, a novel glial fibrillary acidic protein isoform," *Glia*, **55**, No. 5, 497-507 (2007).
32. W. Kamphuis, C. Mamber, M. Moeton, 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*, **7**, No. 8, e42823 (2012).
33. E. M. Hol, R. F. Roelofs, E. Moraal, et al., "Neuronal expression of GFAP in patients with Alzheimer pathology and identification of novel GFAP splice forms," *Mol. Psychiat.*, **8**, No. 9, 786-796 (2003).
34. M. Prust, J. Wang, H. Morizono, et al., "GFAP mutations, age at onset, and clinical subtypes in Alexander disease," *Neurology*, **77**, No. 13, 1287-1294 (2011).
35. R. A. Quinlan, M. Brenner, J. E. Goldman, and A. Messing, "GFAP and its role in Alexander disease," *Exp. Cell Res.*, **313**, No. 10, 2077-2087 (2007).
36. J. E. Goldman, H. H. Schaumburg, and W. T. Norton, "Isolation and characterization of glial filaments from human brain," *J. Cell Biol.*, **78**, No. 2, 426-440 (1978).
37. D. Dahl, "Isolation and initial characterization of glial fibrillary acidic protein from chicken, turtle, frog and fish central nervous systems," *Biochim. Biophys. Acta*, **446**, No. 1, 41-50 (1976).
38. M. Sancho Tello, S. Valles, C. Montoliu, et al., "Developmental pattern of GFAP and vimentin gene expression in rat brain and in radial glial cultures," *Glia*, **15**, No. 2, 156-166 (1995).
39. C. F. Landry, G. O. Ivy, and I. R. Brown, "Developmental expression of glial fibrillary acidic protein mRNA in the rat brain analyzed by *in situ* hybridization," *J. Neurosci. Res.*, **25**, No. 2, 194-203 (1990).
40. F. C. Gomes, D. Paulin, and V. Moura Neto, "Glial fibrillary acidic protein (GFAP): modulation by growth factors and its implication in astrocyte differentiation," *Braz. J. Med. Biol. Res.*, **32**, No. 5, 619-631 (1999).
41. U. Wilhelmsson, C. Eliasson, R. Bjerkvig, and M. Pekny, "Loss of GFAP expression in high-grade astrocytomas does not contribute to tumor development or progression," *Oncogene*, **22**, 3407-3411 (2003).
42. A. Zamoner, C. Funchal, M. C. Jacques-Silva, et al., "Thyroid hormones reorganize the cytoskeleton of glial cells through GFAP phosphorylation and Rhoa-dependent mechanisms," *Cell Mol. Neurobiol.*, **27**, No. 7, 845-865 (2007).
43. S. Brahmachari, Y. K. Fung, and K. Pahan, "Induction of glial fibrillary acidic protein expression in astrocytes by nitric oxide," *J. Neurosci.*, **26**, No. 18, 4930-4939 (2006).
44. J. Guo, D. Jia, and B. Jin, "Effects of glial cell line-derived neurotrophic factor intrathecal injection on spinal dorsal horn glial fibrillary acidic protein expression in a rat model of neuropathic pain," *Int. J. Neurosci.*, **122**, No. 7, 388-394 (2012).
45. N. J. Laping, B. Teter, N. R. Nichols, et al., "Glial fibrillary acidic protein: regulation by hormones, cytokines, and growth factors," *Brain Pathol.*, **4**, No. 3, 259-275 (1994).
46. A. Michelucci, A. Bithell, M. J. Burney, et al., "The neurogenic potential of astrocytes is regulated by inflammatory signals," *Mol. Neurobiol.*, (2015).
47. A. L. Nielsen and A. L. Jørgensen, "Self-assembly of the cytoskeletal glial fibrillary acidic protein is inhibited by an isoform-specific C terminus," *J. Biol. Chem.*, **279**, No. 40, 41537-41545 (2004).
48. M. Kornreich, R. Avinery, E. Malka-Gibor, et al., "Order and disorder in intermediate filament proteins," *FEBS*

Lett., **589**, 19 Part A, 2464-2476 (2015).
49. R. L. Shoeman and P. Traub, "Assembly of intermediate filaments," *BioEssay*, **15**, No. 9, 605-611 (1993).
50. M. Inagaki, Y. Nakamura, M. Takeda, et al., "Glial fibrillary acidic protein: dynamic property and regulation by phosphorylation," *Brain Pathol.*, **4**, No. 3, 239-243 (1994).
51. M. Garbuglia, M. Verzini, and R. Donato, "Annexin VI binds S100A1 and S100B and blocks the ability of S100A1 and S100B to inhibit desmin and GFAP assemblies into intermediate filaments," *Cell Calcium*, **24**, No. 3, 177-191 (1998).
52. D. G. Graham, "Neurotoxicants and the cytoskeleton," *Curr. Opin. Neurol.*, **12**, No. 6, 733-737 (1999).
53. V. S. Nedzvetskiĭ, G. A. Ushakova, S. G. Busygina, et al., "The effect of low doses of ionizing radiation on the intermediate filaments and the Ca^{2+}-activated proteolysis system in the rat brain," *Radiobiologiia*, **31**, No. 3, 333-339 (1991).
54. T. T. Rohn, L. W. Catlin, and W. W. Poon, "Caspase-cleaved glial fibrillary acidic protein within cerebellar white matter of the Alzheimer's disease brain," *Int. J. Clin. Exp. Pathol.*, **6**, No. 1, 41-48 (2013).
55. G. Baydas, R. J. Reiter, V. S. Nedzvetskii, et al., "Altered glial fibrillary acidic protein content and its degradation in the hippocampus, cortex and cerebellum of rats exposed to constant light: reversal by melatonin," *J. Pineal Res.*, **33**, No. 3, 134-139 (2002).
56. V. S. Nedzvetskiĭ, S. G. Busygina, V. A. Berezin, and A. I. Dvoretskiĭ, "The CNS syndrome. The characteristics of the intermediate filaments of the rat brain," *Radiobiologiia*, **30**, No. 2, 243-246 (1990).
57. H. Zetterberg, D. H. Smith, and K. Blennow, "Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood," *Nat. Rev. Neurol.*, **9**, No. 4, 201-210 (2013).
58. A. M. Boutté, Y. Deng-Bryant, D. Johnson, et al., "Serum glial fibrillary acidic protein predicts tissue glial fibrillary acidic protein break-down products and therapeutic efficacy after penetrating ballistic-like brain injury," *J. Neurotrauma*, **33**, No. 1, 147-156 (2016).
59. J. W. Bigbee, D. D. Bigner, C. Pegram, and L. F. Eng, "Study of glial fibrillary acidic protein in a human glioma cell line grown in culture and as a solid tumor," *J. Neurochem.*, **40**, No. 2, 460-467 (1983).
60. M. Takemura, H. Gomi, E. Colucci-Guyon, and S. Itohara, "Protective role of phosphorylation in turnover of glial fibrillary acidic protein in mice," *J. Neurosci.*, **22**, No. 16, 6972-6979 (2002).
61. N. T. Snider and M. B. Omary, "Assays for posttranslational modifications of intermediate filament proteins," *Methods Enzymol.*, **568**, 113-138 (2016).
62. J. H. Herskowitz, N. T. Seyfried, and D. M. Duong, "Phosphoproteomic analysis reveals site-specific changes in GFAP and NDRG2 phosphorylation in frontotemporal lobar degeneration," *J. Proteome Res.*, **9**, No. 12, 6368-6379 (2010).
63. R. Rodnight, C. A. Gonçalves, S. T. Wofchuk, and R. Leal, "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.*, **30**, No. 3, 325-338 (1997).
64. N. T. Snider and M. B. Omary, "Post-translational modifications of intermediate filament proteins: mechanisms and functions," *Nat. Rev. Mol. Cell Biol.*, **15**, No. 3, 163-177 (2014).
65. S. M. Sullivan, R. K. Sullivan, S. M. Miller, et al., "Phosphorylation of GFAP is associated with injury in the neonatal pig hypoxic-ischemic brain," *Neurochem. Res.*, **37**, No. 11, 2364-2378 (2012).
66. Z. Jin, Z. Fu, J. Yang, et al., "Identification and characterization of citrulline-modified brain proteins by combining HCD and CID fragmentation," *Proteomics*, **13**, No. 17, 2682-2691 (2013).
67. D. Liu, C. Liu, J. Li, et al., "Proteomic analysis reveals differentially regulated protein acetylation in human amyotrophic lateral sclerosis spinal cord," *PLoS One*, **8**, No. 12, e80779 (2013).
68. S. J. DeArmond, M. Fajardo, S. A. Naughton, and L. F. Eng, "Degradation of glial fibrillary acidic protein by a calcium dependent proteinase: an electroblot study," *Brain Res.*, **262**, No. 2, 275-282 (1983).
69. Y. B. Lee, S. Du, H. Rhim, 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 Res.*, **864**, No. 2, 220-229 (2000).
70. P. E. Mouser, E. Head, K. H. Ha, et al., "Caspase-mediated cleavage of glial fibrillary acidic protein within degenerating astrocytes of the Alzheimer's disease brain," Am. J. Pathol., 168, No. 3, 936-946 (2006).
71. M. H. Chen, T. L. Hagemann, R. A. Quinlan, et al., "Caspase cleavage of GFAP produces an assembly-compromised proteolytic fragment that promotes filament aggregation," *ASN Neuro*, **5**, No. 5, e00125 (2013).
72. L. Li, J. V. Welser, P. Dore-Duffy, et al., "In the hypoxic central nervous system, endothelial cell proliferation is followed by astrocyte activation, proliferation, and increased expression of the alpha 6 beta 4 integrin and dystroglycan," *Glia*, **58**, No. 10, 1157-1167 (2010).
73. M. Pekny, P. Levéen, M. Pekna, et al., "Mice lacking glial fibrillary acidic protein display astrocytes devoid of intermediate filaments but develop and reproduce normally," *EMBO J.*, **14**, No. 8, 1590-1598.
74. W. Liedtke, W. Edelmann, P. L. Bieri, et al., "GFAP is necessary for the integrity of CNS white matter architecture and long-term maintenance of myelination," *Neuron*, **17**, No. 4, 607-615 (1996).
75. W. Liedtke, W. Edelmann, F. C. Chiu, et al., "Experimental autoimmune encephalomyelitis in mice lacking glial fibrillary acidic protein is characterized by a more severe clinical course and an infiltrative central nervous system lesion," *Am. J. Pathol.*, **152**, No. 1, 251-259 (1998).
76. R. Tian, X. Wu, T. L. Hagemann, et al., "Alexander disease mutant glial fibrillary acidic protein compromises glutamate transport in astrocytes," *J. Neuropathol. Exp. Neurol.*, **69**, No. 4, 335-345 (2010).
77. M. Tardy, C. Fages, G. Le Prince, et al., "Regulation

of the glial fibrillary acidic protein (GFAP) and of its encoding mRNA in the developing brain and in cultured astrocytes," *Adv. Exp. Med. Biol.*, **265**, 41-52 (1990).
78. M. A. McCall, R. G. Gregg, R. R. Behringer, et al., "Targeted deletion in astrocyte intermediate filament (GFAP) alters neuronal physiology," *Proc. Natl. Acad. Sci. USA*, **93**, No. 13, 6361-6366 (1996).
79. M. Pekny, C. Eliasson, and C. L. Chien, "GFAP-deficient astrocytes are capable of stellation *in vitro* when cocultured with neurons and exhibit a reduced amount of intermediate filaments and an increased cell saturation density," *Exp. Cell Res.*, **239**, No. 2, 332-343 (1998).
80. M. V. Sofroniew, "Astrogliosis," *Cold Spring Harb. Perspect. Biol.*, **7**, No. 2, a020420 (2014).
81. M. Pekny, U. Wilhelmsson, and M. Pekna, "The dual role of astrocyte activation and reactive gliosis," *Neurosci. Lett.*, **565**, 30-38 (2014).
82. R. S. Ghirnikar, A. C. Yu, and L. F. Eng, "Astrogliosis in culture: III. Effect of recombinant retrovirus expressing antisense glial fibrillary acidic protein RNA," *J. Neurosci. Res.*, **38**, No. 4, 376-385 (1994).
83. D. Triolo, G. Dina, I. Lorenzetti, et al., "Loss of glial fibrillary acidic protein (GFAP) impairs Schwann cell proliferation and delays nerve regeneration after damage," *J. Cell Sci.*, **119**, Part 19, 3981-3993 (2006).
84. M. Sugaya-Fukasawa, T. Watanabe, M. Tamura, et al., "Glial fibrillary acidic protein is one of the key factors underlying neuron-like elongation in PC12 cells," *Exp. Ther. Med.*, **2**, No. 1, 85-87 (2011).
85. J. H. Kim, S. J. Kwon, M. C. Stankewich, et al., "Reactive protoplasmic and fibrous astrocytes contain high levels of calpain-cleaved alpha 2 spectrin," *Exp. Mol. Pathol.*, **100**, No. 1, 1-7 (2016).
86. S. Safavi-Abbasi, J. R. Wolff, and M. Missler, "Rapid morphological changes in astrocytes are accompanied by redistribution but not by quantitative changes of cytoskeletal proteins," *Glia*, **36**, No. 1, 102-115 (2001).
87. B. D. Gulbransen and K. A. Sharkey, "Novel functional roles for enteric glia in the gastrointestinal tract," *Nat. Rev. Gastroenterol. Hepatol.*, **9**, No. 11, 625-632 (2012).
88. G. B. von Boyen, M. Steinkamp, M. Reinshagen, et al., "Proinflammatory cytokines increase glial fibrillary acidic protein expression in enteric glia," *Gut*, **53**, No. 2, 222-228 (2004).
89. C. Laranjeira, K. Sandgren, N. Kessaris, et al., "Glial cells in the mouse enteric nervous system can undergo neurogenesis in response to injury," *J. Clin. Invest.*, **121**, No. 9, 3412-3424 (2011).
90. EPA/630/R-95/001, "Guidelines for neurotoxicity risk assessment," *Fed. Register,* **63**, 26926-26954 (1998).
91. G. Baydas, V. S. Nedzvetskii, M. Tuzcu, et al., "Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: effects of vitamin E," *Eur. J. Pharmacol.*, **462**, Nos. 1/3, 67-71 (2003).
92. V. S. Nedzvetsky, M. Tuzcu, A. Yasar, et al., "Effects of vitamin E against aluminum neurotoxicity in rats," *Biochemistry*, **71**, No. 3, 239-244 (2006).
93. K. Kaneko, A. Nakamura, K. Yoshida, et al., "Glial fibrillary acidic protein is greatly modified by oxidative stress in aceruloplasminemia brain," *Free Radical Res.*, **36**, No. 3, 303-306 (2002).
94. C. S. Chiang, W. H. McBride, and H. R. Withers, "Radiation-induced astrocytic and microglial responses in mouse brain," *Radiother. Oncol.*, **29**, No. 1, 60-68 (1993).
95. M. Carballo-Quintás, I. Martínez-Silva, C. Cadarso-Suárez, et al., "A study of neurotoxic biomarkers, c-fos and GFAP after acute exposure to GSM radiation at 900 MHz in the picrotoxin model of rat brains," *Neurotoxicology*, **32**, No. 4, 478-494 (2011).
96. D. Schiffer and V. Fiano, "Astrogliosis in ALS: possible interpretations according to pathogenetic hypotheses," *Amyotroph. Lateral. Scler. Other Motor. Neuron. Disord.*, **5**, No. 1, 22-25 (2004).
97. E. C. Hirsch, T. Breidert, E. Rousselet, et al., "The role of glial reaction and inflammation in Parkinson's disease," *Ann. N.Y. Acad. Sci.*, **991**, 214-228 (2003).
98. K. L. Goodison, I. M. Parhad, C. L. White, et al., "Neuronal and glial gene expression in neocortex of Down's syndrome and Alzheimer's disease," *J. Neuropathol. Exp. Neurol.*, **52**, No. 3, 192-198 (1993).
99. G. W. Ross, J. P. O'Callaghan, and D. S. Sharp, "Quantification of regional glial fibrillary acidic protein levels in Alzheimer's disease," *Acta Neurol. Scand.*, **107**, No. 5, 318-323 (2003).
100. S. S. Panter, J. D. McSwigan, and I. R. Sheppard, "Glial fibrillary acidic protein and Alzheimer's disease," *Neurochem. Res.*, **10**, No. 12, 1567-1576 (1985).
101. G. Levi, M. Patrizio, A. Bernardo, et al., "Human immunodeficiency virus coat protein gp120 inhibits the beta-adrenergic regulation of astroglial and microglial functions," *Proc. Natl. Acad. Sci. USA*, **90**, No. 4, 1541-1545 (1993).
102. P. G. Kennedy, E. O. Major, R. K. Williams, and S. E. Straus, "Down-regulation of glial fibrillary acidic protein expression during acute lytic varicella-zoster virus infection of cultured human astrocytes," *Virology*, **205**, No. 2, 558-562 (1994).
103. L. Rinaman, J. P. Card, and L. W. Enquist, "Spatiotemporal responses of astrocytes, ramified microglia, and brain macrophages to central neuronal infection with pseudorabies virus," *J. Neurosci.*, **13**, No. 2, 685-702 (1993).
104. S. E. Arnold, B. R. Franz, J. Q. Trojanowski, et al., "Glial fibrillary acidic protein-immunoreactive astrocytosis in elderly patients with schizophrenia and dementia," *Acta Neuropathol.*, **91**, No. 3, 269-277 (1996).
105. E. Danzer, L. Zhang, A. Radu, et al., "Amniotic fluid levels of glial fibrillary acidic protein in fetal rats with retinoic acid induced myelomeningocele: a potential marker for spinal cord injury," *Am. J. Obstet. Gynecol.*, **204**, No. 2, 178, e1-11 (2011).
106. P. E. Vos, M. van Gils, T. Beems, et al., "Increased GFAP and S100beta but not NSE serum levels after subarachnoid haemorrhage are associated with clinical severity," *Eur. J. Neurol.*, **13**, No. 6, 632-638 (2006).
107. J. Steiner, H. Bielau, H. G. Bernstein, et al., "Increased cerebrospinal fluid and serum levels of S100B in first-

onset schizophrenia are not related to a degenerative release of glial fibrillar acidic protein, myelin basic protein and neurone-specific enolase from glia or neurons," *J. Neurol., Neurosurg., Psychiat.*, **77**, No. 11, 1284-1287 (2006).
108. P. Wei, W. Zhang, L. S. Yang, et al., "Serum GFAP autoantibody as an ELISA-detectable glioma marker," *Tumour Biol.*, **34**, No. 4, 2283-2292 (2013).
109. Z. Zhang, J. S. Zoltewicz, S. Mondello, et al., "Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products," *PLoS One*, **9**, No. 3, e92698 (2014).
110. C. F. Lucchinetti, Y. Guo, B. F. Popescu, et al., "The pathology of an autoimmune astrocytopathy: lessons learned from neuromyelitis optica," *Brain Pathol.*, **24**, No. 1, 83-97 (2014).
111. A. Ishigami, H. Masutomi, S. Handa, et al., "Mass spectrometric identification of citrullination sites and immunohistochemical detection of citrullinated glial fibrillary acidic protein in Alzheimer's disease brains," *J. Neurosci. Res.*, **93**, No. 11, 1664-1674 (2015).
112. A. J. Mehta, "Alcoholism and critical illness: a review," *World J. Crit. Care Med.*, **5**, No. 1, 27-35 (2016).
113. C. J. Wilhelm, J. G. Hashimoto, and M. L. Roberts, et al., "Astrocyte dysfunction induced by alcohol in females but not males," *Brain Pathol.*, **19**, doi: 10.1111/bpa (2015).
114. H. Franke, H. Kittner, P. Berger, et al., "The reaction of astrocytes and neurons in the hippocampus of adult rats during chronic ethanol treatment and correlations to behavioral impairments," *Alcohol*, **14**, No. 5, 445-454 (1997).
115. C. Bull, W. A. Syed, S. C. Minter, and M. S. Bowers, "Differential response of glial fibrillary acidic protein-positive astrocytes in the rat prefrontal cortex following ethanol self-administration," *Alcohol. Clin. Exp. Res.*, **39**, No. 4, 650-658 (2015).
116. K. P. Reis, L. Heimfarth, P. Pierozan, et al., "High postnatal susceptibility of hippocampal cytoskeleton in response to ethanol exposure during pregnancy and lactation," *Alcohol*, **49**, No. 7, 665-674 (2015).
117. S. Vallés, J. Pitarch, J. Renau-Piqueras, and C. Guerri, "Ethanol exposure affects glial fibrillary acidic protein gene expression and transcription during rat brain development," *J. Neurochem.*, **69**, No. 6, 2484-2493 (1997).
118. C. Guerri and J. Renau-Piqueras, "Alcohol, astroglia, and brain development," *Mol. Neurobiol.*, **15**, No. 1, 65-81 (1997).
119. A. A. Tikhomirov, V. S. Nedzvetskii, M. V. Lipka, et al., "Chronic alcoholization-induced damage to astroglia and intensification of lipid peroxidation in the rat brain: protector effect of hydrated form of fullerene C_{60}," *Neurophysiology*, **39**, No. 2, 105-111 (2007).
120. A. A. Tykhomyrov, V. S. Nedzvetsky, V. K. Klochkov, and G. V. Andrievsky, "Nanostructures of hydrated C60 fullerene (C60HyFn) protect rat brain against alcohol impact and attenuate behavioral impairments of alcoholized animals," *Toxicology*, **246**, Nos. 2/3, 158-165 (2008).
121. A. A. Tikhomirov, G. V. Andrievsky, and V. S. Nedzvetsky, "Disorders in the cytoskeleton of astroglia and neurons in the rat brain induced by long-lasting exposure to ethanol and correction of these shifts by hydrated fullerene C_{60}," *Neurophysiology*, **40**, No. 4, 279-287 (2008).
122. A. D. Thomson, "Mechanisms of vitamin deficiency in chronic alcohol misusers and the development of the Wernicke-Korsakoff syndrome," *Alcohol Alcohol. Suppl.*, **35**, No. 1, 2-7 (2000).
123. Y. M. Parkhomenko, P. A. Kudryavtsev, S. Y. Pylypchuk, et al., "Chronic alcoholism in rats induces a compensatory response, preserving brain thiamine diphosphate, but the brain 2-oxo acid dehydrogenases are inactivated despite unchanged coenzyme levels," *J. Neurochem.*, **117**, No. 6, 1055-1065 (2011).
124. A. Sharma, R. Bist, and P. Bubber, "Thiamine deficiency induces oxidative stress in brain mitochondria of Mus musculus," *J. Physiol. Biochem.*, **69**, No. 3, 539-546 (2013).
125. S. S. Karuppagounder, H. Xu, Q. Shi, et al., "Thiamine deficiency induces oxidative stress and exacerbates the plaque pathology in Alzheimer's mouse model," *Neurobiol. Aging*, **30**, No. 10, 1587-1600 (2009).
126. A. S. Hazell, "Astrocytes are a major target in thiamine deficiency and Wernicke's encephalopathy," *Neurochem. Int.*, **55**, Nos. 1/3, 129-135 (2009).
127. A. S. Hazell, K. V. Rao, N. C. Danbolt, et al., "Selective down-regulation of the astrocyte glutamate transporters GLT-1 and GLAST within the medial thalamus in experimental Wernicke's encephalopathy," *J. Neurochem.*, **78**, No. 3, 560-568 (2001).
128. P. Desjardins, K. G. Todd, A. S. Hazell, and R. F. Butterworth, "Increased 'peripheral-type' benzodiazepine receptor sites and mRNA in thalamus of thiamine-deficient rats," *Neurochem. Int.*, **35**, No. 5, 363-369 (1999).
129. S. Afadlal, R. Labetoulle, and A. S. Hazell, "Role of astrocytes in thiamine deficiency," *Met. Brain Dis.*, **29**, No. 4, 1061-1068 (2014).
130. R. Peters, "Ageing and the brain," *Postgrad. Med. J.*, **82**, No. 964, 84-88 (2006).
131. J. P. David, F. Ghozali, C. Fallet-Bianco, et al., "Glial reaction in the hippocampal formation is highly correlated with aging in human brain," *Neurosci. Lett.*, **235**, Nos. 1/2, 53-56 (1997).
132. J. A. Sloane, W. Hollander, D. L. Rosene, et al., "Astrocytic hypertrophy and altered GFAP degradation with age in subcortical white matter of the rhesus monkey," *Brain Res.*, **862**, Nos. 1/2, 1-10 (2000).
133. Y. Wu, A. Q. Zhang, and D. T. Yew, "Age-related changes of various markers of astrocytes in senescence-accelerated mice hippocampus," *Neurochem. Int.*, **46**, No. 7, 565-574 (2005).
134. M. Sabbatini, P. Barili, E. Bronzetti, et al., "Age-related changes of glial fibrillary acidic protein immunoreactive astrocytes in the rat cerebellar cortex," *Mech. Ageing Dev.*, **108**, No. 2, 165-172 (1999).
135. I. Jalenques, A. Burette, E. Albuisson, and R. Romand, "Age-related changes in GFAP-immunoreactive

astrocytes in the rat ventral cochlear nucleus," *Hear. Res.*, **107**, Nos. 1/2, 113-124 (1997).
136. M. T. Berciano, M. A. Andres, E. Calle, and M. Lafarga, "Age-induced hypertrophy of astrocytes in rat supraoptic nucleus: a cytological, morphometric, and immunocytochemical study," *Anat. Rec.*, **243**, No. 1, 129-144 (1995).
137. H. J. Jyothi, D. J. Vidyadhara, A. Mahadevan, et al., "Aging causes morphological alterations in astrocytes and microglia in human *substantia nigra pars compacta*," *Neurobiol. Aging*, **36**, No. 12, 3321-3333 (2015).
138. S. Sharma, T. C. Nag, A. Thakar, et al., "The aging human cochlear nucleus: changes in the glial fibrillary acidic protein, intracellular calcium regulatory proteins, GABA neurotransmitter and cholinergic receptor," *J. Chem. Neuroanat.*, **56**, 1-12 (2014).
139. A. Catalani, M. Sabbatini, C. Consoli, et al., "Glial fibrillary acidic protein immunoreactive astrocytes in developing rat hippocampus," *Mech. Ageing Dev.*, **123**, No. 5, 481-490 (2002).
140. F. Amenta, E. Bronzetti, M. Sabbatini, and J. A. Vega, "Astrocyte changes in aging cerebral cortex and hippocampus: a quantitative immunohistochemical study," *J. Microscop. Res. Tech.*, **43**, No. 1, 29-33 (1998).
141. N. Hayakawa, H. Kato, and T. Araki, "Age-related changes of astorocytes, oligodendrocytes and microglia in the mouse hippocampal *CA1* sector," *Mech. Ageing Dev.*, **128**, No. 4, 311-316 (2007).
142. I. K. Hwang, J. H. Choi, H. Li, et al., "Changes in glial fibrillary acidic protein immunoreactivity in the dentate gyrus and hippocampus proper of adult and aged dogs," *J. Vet. Med. Sci.*, **70**, No. 9, 965-969 (2008).
143. C. E. Finch, "Neurons, glia, and plasticity in normal brain aging," *Adv. Gerontol.*, **10**, 35-39 (2002).
144. A. Latour, B. Grintal, G. Champeil-Potokar, et al., "Omega-3 fatty acids deficiency aggravates glutamatergic synapse and astroglial aging in the rat hippocampal *CA1*," *Aging Cell*., **12**, No. 1, 76-84 (2013).
145. J. R. Day, A. T. Frank, J. P. O'Callaghan, et al., "The effect of age and testosterone on the expression of glial fibrillary acidic protein in the rat cerebellum," *Exp. Neurol.*, **151**, No. 2, 343-346 (1998).
146. C. P. Anderson, I. Rozovsky, D. J. Stone, et al., "Aging and increased hypothalamic glial fibrillary acid protein (GFAP) mRNA in F344 female rats. Dissociation of GFAP inducibility from the luteinizing hormone surge," *Neuroendocrinology*, **76**, No. 2, 121-130 (2002).