===== REVIEW =====

Astrocyte Activation Markers

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Abstract—Astrocytes are the most common type of glial cells that provide homeostasis and protection of the central nervous system. Important specific characteristic of astrocytes is manifestation of morphological heterogeneity, which is directly dependent on localization in a particular area of the brain. Astrocytes can integrate into neural networks and keep neurons active in various areas of the brain. Moreover, astrocytes express a variety of receptors, channels, and membrane transporters, which underlie their peculiar metabolic activity, and, hence, determine plasticity of the central nervous system during development and aging. Such complex structural and functional organization of astrocytes requires the use of modern methods for their identification and analysis. Considering the important fact that determining the most appropriate marker for polymorphic and multiple subgroups of astrocytes is of decisive importance for studying their multifunctionality, this review presents markers, modern imaging techniques, and identification of astrocytes, which comprise a valuable resource for studying structural and functional properties of astrocytes, as well as facilitate better understanding of the extent to which astrocytes contribute to neuronal activity.

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INTRODUCTION

Astrocytes have long been considered as auxiliary cells solely ensuring trophic, metabolic, and structural support to neurons [1]. However, over the last three decades extensive studies demonstrated that they play a pivotal role in physiological and pathological functions of the brain. In particular, astrocytes regulate ion homeostasis, cerebral blood flow, contribute to neovascularization, protect from excitotoxic neuronal injury and death, promote synapse formation, and participate in cerebral energy metabolism and development of the blood-brain barrier (BBB) [2].

Moreover, along with the pre- and post-synaptic membranes astrocytes form the so-called tripartite synapse thereby ensuring efficient synaptic transmission [3]. Upon that, astrocytes secrete gliotransmitters, cytokines, and metabolites participating in the neuron-astroglia coupling and gliovascular control [4].

In addition, it is also worth noting that astrocytes are able to modulate microglia phenotype and phagocytosis via crosstalk between astrocytes and microglia implying release of diverse signaling molecules by both cell types allowing to establish an autocrine feedback loop for targeted reciprocal modulation upon damage of the central nervous system (CNS) [5].

Abbreviations: AQP4, aquaporin 4; CaM, calmodulin; ChR2, channelrhodopsin-2; Cx, connexin; EGFP, enhanced green fluorescent protein; GFP, green fluorescent protein; GFAP, glial fibrillar acidic protein; GS, glutamine synthetase; *NDRG2*, N-myc downstream-regulated gene 2.

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Overall, astrocytes represent a crucial element in the cerebral development and functioning [6]. Astrocyte activity is based on the dynamic intra- and inter-cellular interactions regulating synaptic plasticity, interaction in neuronal circuits, as well as contributing to memory consolidation [7]. Being a pivotal component of astrocyte activity, fluctuations of the intracellular free Ca^{2+} could occur spontaneously or due to neurotransmitter-mediated activation of the NMDARs (N-methyl-D-aspartate receptors) and TRP (Transient receptor potential) channels as well as GPCRs (G-protein-coupled receptors) [8]. Such astrocyte signaling mediated by elevated cytosolic Ca^{2+} concentration ensures gliotransmitter release, neurovascular interaction, as well as presynaptic structural remodeling [9]. Recent evidence [10] demonstrates that the astroglial type 1 cannabinoid receptors (CB1) determine availability of the hippocampal synaptic D-serine (synaptic NMDAR agonist). Hence, it demonstrates that the astroglial CB1 receptors act as a key component underlying physiological object recognition memory consolidation in hippocampus. For instance, the *CB1* gene ablation results in the impaired D-serine binding to hippocampal synaptic NMDAR in the GFAP-*CB1*-knockout mice, which is associated with the impaired object recognition memory as well as decreased long-term potentiation at hippocampal synapses. It suggests a novel mechanism for astroglial regulation of synaptic plasticity via the D-serine-dependent control of NMDARs [10].

It should be also noted that heterogeneous gene expression, morphology, and functional interactions with local neuronal circuits is typical for astrocytes [11]. Neuron—astrocyte interaction is necessary for enabling diverse events such as synapse formation and functioning, neurotransmitter release and reuptake, production of neurophysiological cues, as well as control of neuronal survival. It must be mentioned in this regard that the functional neuron-astrocyte crosstalk is largely sustained both in adult and aging brain of the healthy animals, but becomes profoundly altered in the process of development of neurodegenerative diseases [12].

Obviously, visualization and identification of astrocytes, especially in the *in situ* specimens and in the brain *in vivo* is a challenging task. In this case, the problems are associated with the remarkable morphological heterogeneity and lack of the universal astrocyte marker able to reveal all astroglial lineage cells. Existing methods include classical histological staining and immunocytochemistry (performed on fixed tissues), genetically-controlled expression of the astrocyte-specific fluorescent markers, incubation with fluorescent probes, as well as intra-glial fluorescent dye injection.

Here, we discuss various specific molecular markers as well as current visualization and identification approaches for astrocytes characterized by polymorphism and multifunctionality, which might provide an opportunity to extend our understanding of the astrocyte

contribution to neuronal activity, modulated synaptic transmission and plasticity, as well as bidirectional neuron-astrocyte crosstalk.

DIVERSITY OF ASTROCYTE MARKERS

Until recently, no universal marker able to stain and identify astrocytes in the CNS has been proposed, which is due to inherent morphological heterogeneity of astrocyte, which, in turn, coupled with the remarkably diverse expression of various molecules [13] (table).

Glial fibrillar acidic protein (GFAP). Glial fibrillar acidic protein (GFAP) is one of the widely recognized astrocyte markers, which is the major intermediate filament protein found in mature astrocytes and crucial cytoskeletal constituent in the developing astrocytes [14]. It is worth emphasizing that GFAP as a common astrocyte-specific marker is mainly expressed in the white matter rather than in the grey matter astrocytes [44]. Moreover, GFAP is unable to fully delineate all astrocyte processes, 85% of which were negative for this marker [45].

Remarkably, under pathophysiological settings the upregulated GFAP expression serves as a sensitive and reliable reactive astrocyte marker, whereas in the intact tissue numerous astrocytes expressed it below the threshold of immunohistochemical detection, which, therefore, indicates that lack of the detected GFAP-positive signal does not allow to conclude that no astrocytes are present [16].

Moreover, it is also interesting that astrocytes in various cerebral areas may differ remarkably in the level of GFAP expression so that in the hippocampus, but not in the thalamus, it could be upregulated [14, 15].

It was found that in the healthy human brain GFAP was mainly expressed in mature astrocytes [14]. However, experimental studies revealed that GFAP expression was observed in the radial glia of the developing human brain as well as adult neural stem cells [15, 46], thereby suggesting that GFAP is also expressed in immature undifferentiated cells in the CNS (figure).

Furthermore, it was additionally demonstrated in the study aimed at investigating mouse cortical somatosensory cells and hippocampal CA1 region using the single-cell RNA sequencing (scRNA-Seq) [47] that they contained 47 molecularly distinct subclasses including all known major cell types in the cortex. Among them, astrocytes formed only two subclasses, one of which contained GFAP-positive astrocytes, whereas another — astrocytes expressing milk fat globule epidermal growth factor 8 (Mfge8), suggesting again limited GFAP expression in the substantial portion of astroglial cells. Moreover, it is important to take into account that GFAP is expressed in the radial glial cells (indeed being stem cells of the CNS), therefore, showing that GFAP detection in neurogenesis brain regions does not correspond to the position of mature astrocytes, which necessitates application of alternative markers.

Astrocyte markers

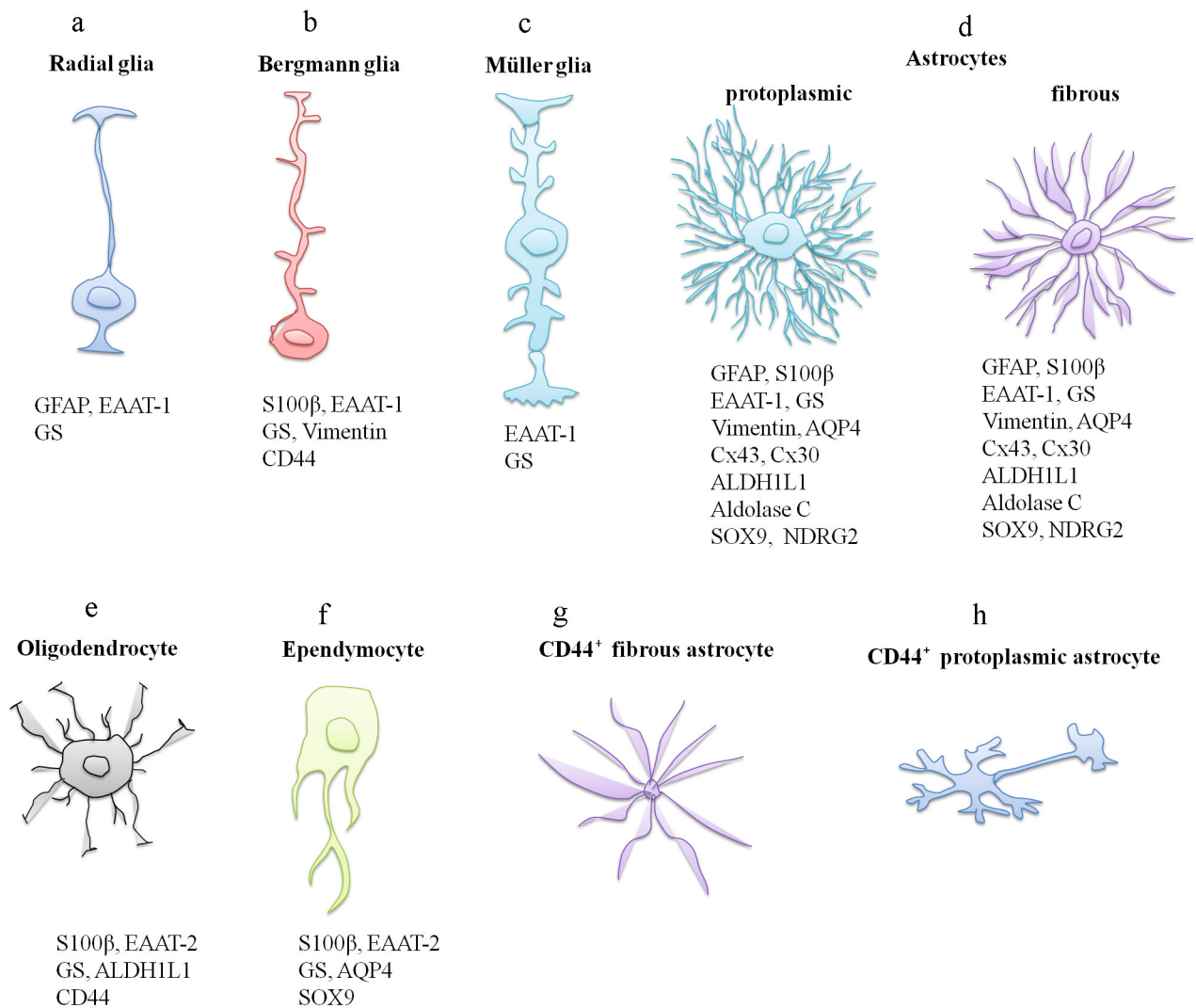| Marker	Protein-related functional role	Cell expression	Local brain expression	Ontogenetic changes	Pathologic changes	References
Glial fibrillar acidic protein (GFAP)	intermediate filament protein; ensures structural stability of the cell; regulates astrocyte morphology and functions	mature astrocytes, radial glia, neural stem cells	brain cortex, hippocampus, amygdala, hypothalamus, olfactory bulb, cerebellum	highly expressed after birth, sustained in aging	high expression level in reactive astrocytes	[14-16]
S100β	maintenance of Ca^{2+} homeostasis; regulates cell proliferation and differentiation; participates in astrocyte intermediate filaments remodeling; biomarker of apoptosis	astrocytes, Bergmann glia, oligodendrocytes, ependymocytes, choroid plexus epithelium, vascular endothelial cells, lymphocytes, neurons	brain cortex, hippocampus, hypothalamus, amygdala, cerebellum	expression level increases in ontogenesis	high expression level in reactive astrocytes	[17-19]
Glutamate transporters: EAAT-1 (GLAST), EAAT-2 (GLT-1)	astroglial specific glutamate transporters; involved in glutamate clearance	EAAT-1 — radial glia, fibrous and protoplasmic astrocytes, Bergmann glia, Müller glia; EAAT-2 — neurons, glial cells (oligodendrocytes, ependymal cells, choroid plexus epithelial cells, satellite cells and Schwann cells)	EAAT-1 — cerebellum; EAAT-2 — hippocampus, lateral septum, brain cortex, striatum	expression level increases in ontogenesis	low expression level	[20, 21]
Glutamine synthetase (GS)	participates in formation of glutamine from ammonia and glutamate intermediate	radial glia, Bergmann glia, retinal Müller glia, astrocytes, ependymal cells, oligodendrocytes, neurons	ubiquitously found across the brain	high activity declines in aging	activity declines	[22, 23]
Vimentin	intermediate filament protein involved in regulating cell differentiation, adhesion, migration, signaling, cellular stress response in differentiating cells	astrocytes, Bergmann glia, adult neural stem cells	brain cortex, cerebellum, hippocampus	expression level decreases in ontogenesis	highly expressed in hypertrophic astrocytes, reactive microglia, macrophages	[24-26]
Aquaporin 4 (AQP4)	a member of the aquaporin protein family, forms water-transporting channels in cell membranes; controls water and ion homeostasis; participates in astrocytes migration, transmission of nerve signals	astrocytes, ependymocytes	heterogeneously expressed across entire CNS: at high levels in cerebellum; at lowest levels in hippocampus and brain cortex	expression level increases in ontogenesis	low expression level	[27, 28]

Marker	Protein-related functional role	Cell expression	Local brain expression	Ontogenetic changes	Pathologic changes	References
Connexins: Cx43, Cx30	organize astrocytic circuits to coordinate activity of local neural networks by transporting glutamate or glutamine; exert a trophic function for neurons by mediating glucose and lactic acid delivery	Cx30 selectively expressed in the grey matter astrocytes, Cx43 and Cx30 co-expressed in astrocyte gap junction	brain cortex, hypothalamus, thalamus and hippocampus	high activity of Cx43 after birth and in aging; rise in Cx30 activity within the first 3 weeks after birth, peaking at 4 weeks postnatally	high expression level in reactive astrocytes	[7, 29, 30]
ALDH1L1	involved in folic acid metabolism	astrocytes, oligodendrocytes	broadly expressed across the brain	expression level decreases in ontogenesis	high expression level in reactive astrocytes	[31-33]
Aldolase C	an enzyme involved in astrocyte glycolysis, providing lactate as a neuronal energy source	astrocytes, cerebellar neurons (Purkinje cells)	cerebellum, brain cortex	highly expressed after birth, sustained in ontogenesis	high expression level in Purkinje cells	[34-36]
SOX9	a transcription factor playing an important role in gene transcription of mature astrocytes as well as regulating functions thereof	astrocytes, neuronal progenitor cells, ependymal cells	hippocampus, olfactory bulb, cerebellum	no decline in expression level in aging	high expression level in reactive astrocytes	[37, 38]
CD44	transmembrane glycoprotein involved in cell−matrix adhesion and matrix-mediated signaling	astrocytes, oligodendrocytes, neural stem cells, Bergmann glia, Purkinje neurons	hippocampus, brain cortex, cerebellum	expression level declines in ontogenesis	high expression level in reactive astrocytes	[39-41]
NDRG2	tumor suppressor and the gene associated with cell stress, cell proliferation and differentiation	mature astrocytes	brain cortex, olfactory bulb, hippocampus, midbrain and thalamus	expression level rises in ontogenesis	high expression level in reactive astrocytes	[42, 43]

S100β. Glycoprotein S100β is a Ca^{2+}-binding protein regulating cell proliferation and differentiation [48]. It was shown that astrocytes isolated from the *S100β*-knockout mice exhibited markedly impaired Ca^{2+} signaling that indicated crucial role played by S100β in maintaining the Ca^{2+} homeostasis in astrocytes [49].

Noteworthy is the fact that S100β could participate in the astrocyte intermediate filament remodeling by preventing excessive GFAP polymerization in the presence of micromolar Ca^{2+} concentration, which, in turn, corroborates the idea that the astrocyte intermediate filaments represent dynamic cytoskeletal structures as well as existing cytosolic cues involved in regulating their assembly [50].

It is known that astrocytes produce and express S100β, which provides the expression-level-dependent neuroprotective or neurotoxic effect, stimulates astroglial cell proliferation by promoting its reactivation at higher level, as well as regulates microglia activation at inflammatory sites [51]. Moreover, experimental data suggest that S100β acts as a glial modulator of neuronal synaptic plasticity and long-term potentiation [52].

a
Radial glia

GFAP, EAAT-1
GS

b
Bergmann glia

S100β, EAAT-1
GS, Vimentin
CD44

c
Müller glia

EAAT-1
GS

d
Astrocytes

protoplasmic

GFAP, S100β
EAAT-1, GS
Vimentin, AQP4
Cx43, Cx30
ALDH1L1
Aldolase C
SOX9, NDRG2

fibrous

GFAP, S100β
EAAT-1, GS
Vimentin, AQP4
Cx43, Cx30
ALDH1L1
Aldolase C
SOX9, NDRG2

e
Oligodendrocyte

S100β, EAAT-2
GS, ALDH1L1
CD44

f
Ependymocyte

S100β, EAAT-2
GS, AQP4
SOX9

g
CD44+ fibrous astrocyte

h
CD44+ protoplasmic astrocyte

Glial cells types and relevant visualization markers. a) Radial glia – GFAP expression [77], EAAT-1 [78], GS [77]; b) Bergmann glia – expression of S100β [79], EAAT-1 [80], GS [81], Vimentin [82], CD44 [41]; c) Müller glia – expression of EAAT-1 [83], GS [84]; d) astrocytes – expression of GFAP, S100β, EAAT-1, vimentin, AQP4, ALDH1L1 [85], GS [86], Cx43 [87], Cx30 [88], aldolase C [89], SOX9 [37], NDRG2 [90]; e) oligodendrocytes – expression of S100β [56], EAAT-2 [91], GS [92], ALDH1L1 [93], CD44 [94]; f) ependymocytes – expression of S100β [95], EAAT-2 [96], GS [77], AQP4 [97], SOX9 [98]; g) CD44+ fibrous astrocyte [75]; h) CD44+ protoplasmic astrocyte [75]

In pathological states, S100β expression becomes profoundly altered so that, e.g., its increased serum and cerebrospinal fluid level could be of some diagnostic importance in context of astroglial lineage cell damage [53].

S100β is used as an astrocyte marker due to its high expression level especially in perivascular astroglia. For instance, dual immunofluorescent staining for S100β and GFAP showed that as few as around 80% of the S100β-positive cells were simultaneously positive for GFAP-specific signal, whereas all GFAP-positive cells also co-expressed S100β in the mouse hippocampus. Upon that, the level of expression of S100β in astrocyte was markedly higher than of GFAP. Moreover, the GFAP-positive cells expressing S100β virtually did not differ in size and shape, as well as in S100β-specific immunoreactivity from the GFAP-negative cells [54] (figure).

Furthermore, comparative analysis of the distribution and expression of the three astrocyte-coupled markers such as NDRG2, GFAP, and S100β in the cortex, hippocampus, and thalamus revealed that in comparison with the GFAP-immunopositive astrocytes, NDRG2- and S100β-expressing astrocytes were distributed more evenly across the entire brain. High level of expressed NDRG2 and S100β was observed in the cortex and thalamus, whereas for GFAP it was found in the hippocampus [55].

In addition to this, S100β is expressed in astrocytes both in the white matter and grey matter [56], whereas GFAP was solely observed in the white matter [57]. However, the cell type-specificity of S100β expression was prominently inferior to that of GFAP. In particular, apart from astrocytes, S100β expression has been also observed in oligodendrocytes [17], ependymocytes [18], vascular

endothelium, lymphocytes [19] as well as neurons [58] (figure).

S100a10 is a member of S100 protein family expressed in multiple organs (heart, kidneys, liver, lungs, spleen, gastrointestinal tract), including brain, where it plays an important role in transmembrane trafficking, vesicle secretion, and endocytosis [59]. Unlike the expression of S100β, the S100a10 expression was observed not only in neurons, vascular endothelial cells, astrocytes, microglia, oligodendrocytes, ependymocytes of diverse cerebral regions, but also in pericytes [60]. Furthermore, neuronal expression of S100a10 even within a single cerebral region was less pronounced, whereas higher expression level was observed in astrocytes and microglia. Moreover, S100a10 expression was significantly increased in the reactive astrocytes upon mouse cerebral damage in the stroke and neuroinflammation models [61].

EAAT-1 and EAAT-2. It was found out that glutamate transporters EAAT-1 (GLAST) [21] and EAAT-2 (GLT-1) [21] were almost exclusively expressed in astrocytes. Moreover, EAAT-1 is the most common marker due to its quite abundant localization in the Bergmann glia, Müller glia, and radial glia [62] (figure). High level of GLT-1 expression was found in the hippocampus, lateral septum, cortex and striatum, whereas GLAST was primarily observed in the cerebellum [21].

It is also worth noting that EAAT-1 (GLAST) and EAAT-2 (GLT-1) display no high specificity. In particular, a synthetic peptide GLT-1 was obtained due to alternative splicing that was primarily expressed in the CNS neurons and peripheral nervous system (PNS), but also found in glial cells (oligodendrocytes, ependymal cells, choroid plexus epithelial cells, satellite cells and Schwann cells) (figure). Moreover, immunostaining with antibodies against the synthetic peptide GLT-1 was mainly detected in the neuronal and glial cytosol allowing to assume that GLT-1 could be present in the intracellular vesicle membranes [20].

Glutamine synthetase (GS). Recently, glutamine synthetase (GS), which is expressed in numerous cerebral regions, has been suggested as another astrocyte marker. GS can bind to diverse astrocyte types including radial glia, Müller glia, and Bergmann glia [22] (figure). It is worth noting that the dual immunofluorescent staining of rat hippocampal slices revealed that the GFAP-positive astroglial cells co-expressed GS, thereby composing a GS-positive astrocyte subpopulation, among which around 40% might contain GFAP-negative cells [63]. In addition, other study with dual immunofluorescent staining of the mouse entorhinal cortex revealed that 10% of the cells co-expressed GS and GFAP, whereas the GFAP-positive cells comprised 12%, but 78% of the glial cells solely expressed GS [64].

In astrocytes, GS is located in the cytosol [65]. Moreover, *in vitro* study demonstrated that the astrocyte GS could be also found in the vesicle membranes [22].

However, it should be also remembered that the GS expression is observed both in astrocytes as well as in oligodendrocytes, ependymal cells, and some human brain neurons [23] (figure).

Vimentin. Vimentin is a member of intermediate filament proteins involved in regulating cell migration and differentiation, as well as signal transduction [25]. Vimentin is expressed in astrocytes (figure), and its level could decline gradually in the course of development [66].

It was found out that the mice with ablated *GFAP* and vimentin-coding genes exhibited impaired formation of the glial scars after the CNS damage as well as altered reactivity in the cerebral vasculature [67]. The latter may be due to the pathological change in endothelial and perivascular cell properties (resulting from the loss of vimentin expression therein) [68] and lowered capacity in the surrounding tissues to withstand blood vessel dilation (due to the lack of GFAP and vimentin in astroglial cells). Altogether, it demonstrates that vimentin might be potentially involved in astrocyte activation resulting from the developed changes in CNS.

Aquaporin 4 (AQP4). Another astrocyte marker is aquaporin 4 (AQP4) belonging to the aquaporin protein family, which form water-transporting channels across cell membranes, also attracts certain interest. This protein is specifically upregulated in the astrocytic endfeet, which are in direct contact with the cerebral microvasculature [27]. In addition, it was also found that to a larger extent AQP4 was enriched solely in the astrocytic endfeet processes [69] (figure).

Connexin (Cx). It is worth mentioning that connexin (Cx) expression in astrocytes is observed across the entire brain being dominated by Cx43 over Cx30, with the latter usually colocalized with Cx43 in the astrocyte gap junctions [70]. Moreover, Cx30 is exclusively expressed in the grey matter astrocytes showing extremely heterogeneous distribution [70, 71]. Connexin expression is necessary for formation of astroglial network able to rapidly transmit some low molecular weight substances (lactate, ATP, NAD$^+$) or ions between cells (figure).

ALDH1L1, Aldolase C, and SOX9. ALDH1L1, which is widely expressed across the entire brain in contrast to the traditional astrocyte marker GFAP, was identified as a new highly-specific astroglial marker in the study [33] investigating the genes expressed in the isolated astrocytes, neurons, and oligodendrocytes. It allowed to reveal a highly branched astrocyte morphology including astrocyte cell body and its processes, whereas GFAP primarily labeled the astrocyte processes (figure). ALDH1L1, also known as 10-formyl-tetrahydrofolate dehydrogenase (FDH) [72], is a folic acid metabolism enzyme converting 10-formyl tetrahydrofolate (10-formyl-THF) into tetrahydrofolate, thus playing a crucial role in multiple reactions such as *de novo* nucleotide biosynthesis and methionine regeneration, thereby profoundly affecting cell division and growth [31]. Moreover, there is

evidence regarding a potential link between the aberrant ALDH1L1 expression and neural tube defects during early CNS development [73]. ALDH1L1 expression changes with age so that ALDH1L1 becomes also expressed in oligodendrocyte subpopulation [32]. Moreover, it was discovered that ALDH1L1 mainly marks cortical astrocytes, but, however, displays a rather weak marking of the white matter astrocytes [74].

Enzyme aldolase C (fructose-1,6-bisphosphate aldolase) is expressed to the greater extent in astrocytes, but the respective gene of aldolase C was also found in the cerebellar neurons (Purkinje cells) [35].

The data obtained during the study investigating expression of the transcription factor SOX9 both in mouse and human brain revealed that SOX9 was almost exclusively expressed in the adult astrocytes except ependymal cells and neurogenic niche, where SOX9 was also detected in the neural precursor cells (figure). It is worth mentioning that SOX9 expression was not reduced with aging, but, on the contrary, was enhanced in the reactive astrocytes in the mouse amyotrophic lateral sclerosis model as well as during the middle cerebral artery occlusion. It should be mentioned that SOX9 is an astrocyte-specific nuclear marker that does not allow to fully reveal astrocyte morphology [37].

In addition, the data from another study [75] demonstrated that CD44 is a plasma membrane protein and extracellular matrix receptor allowing to comprehensively examine astrocyte morphology, which, in turn, could be impossible to do by using GFAP. In particular, the CD44-immunopositive astroglial cells with long processes were discovered in the hippocampus and cortex as well as nearby large blood vessels, and phenotype of such astrocytes was shown to be similar to that of the white matter fibrous astrocytes, but was profoundly different from the protoplasmic astrocyte phenotype. At the same time, the CD44-expressing astroglial cells with short processes displaying phenotype similar to that of both protoplasmic and fibrous astrocytes were also detected in the cortex (figure).

In recent years, *NDRG2* (N-myc downstream-regulated gene 2), a tumor suppressor gene related to cell stress, proliferation and differentiation, has been considered as another potential astrocyte marker. It was found out that *NDRG2* is expressed in diverse cerebral regions including cortex, olfactory bulb, hippocampus, midbrain, and thalamus, with the maximum expression being observed in the two latter areas [42]. What is most important is that *NDRG2* was shown to be selectively expressed in the cerebral astrocytes [42, 76]. Moreover, its expression was upregulated in the glioma cells differentiating to astrocytes, whereas in the reactive astrocytes its level was markedly downmodulated [42]. However, some other studies convincingly demonstrated that the expression of *NDRG2* in reactive astrocytes was upregulated in the developing neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [43]. NDRG2 could be related to the glial cell proliferation and differentiation and could be considered as a new astrocyte marker especially for the mature non-reactive cerebral astrocytes (figure).

FLUORESCENT DYES AND PROBES

Fluorescent dyes represent a valuable tool for microscopy because they allow to non-invasively investigate neural cell structure and activity without affecting brain function [99].

In particular, astrocytes could be visualized using fluorescent dyes injected into the cells of interest. Usually, several dyes such as Lucifer yellow, Alexa Fluor, biocytin, Fura-2, etc., are used for cell labeling [45, 99]. For instance, the fluorescent dye Fura-2AM (Fura-2-acetoxymethyl ester) is used for measuring intracellular Ca^{2+} concentration due to its high sensitivity and specificity as well as ability to permeate cell membranes [100].

Alternatively, the Helios Gene Gun transfection system may be used to visualize astrocytes by delivering gold or tungsten particles coated with lipophilic dyes into the live or perfusion fixed brain tissue slices [101]. It allows to rapidly and selectively label cells in various cerebral tissues applying lipophilic dye combinations to facilitate optical separation of closely located cells [102].

Recently, a glyophilic fluorescent probe, cationic dye sulforhodamine 101 and its analogs sulforhodamine B or G, have been suggested for astrocyte visualization [103]. Sulforhodamine undergoes selective uptake by astrocytes and delineates cell structure due to its cytosolic location. Moreover, sulforhodamines could easily penetrate across the blood-brain barrier allowing staining of astrocytes with the intravenously injected sulforhodamine B. Upon that, the astrocyte fluorescence could be observed as early as 40 min post-injection and is sustained for up to 5 h [103]. It seems that in various cerebral regions uptake of sulforhodamine 101 by astrocytes could be mediated by the astrocyte-expressed organic anion transporters. As a result, sulforhodamine 101 efficiently labels hippocampal astrocytes, while it is not accumulated in the astrocytes of the ventrolateral preoptic nucleus [104]. Moreover, it was found out that sulforhodamine 101 selectively labels astrocyte subpopulation of the rat hippocampal slices obtained at early postnatal stage demonstrating typical changes in the course of development as well as traits of the mature GFAP-positive astrocytes [105].

Despite that sulforhodamine 101 efficiently labels astrocytes in the acute rat hippocampal slices [105] as well as mouse cortex *in vivo* [103], it could also stain oligodendrocytes due to activity of the multi-specific carrier OATP1C1 (SLCO1C1) transporting sulforhodamine 101 from astrocytes via gap junctions of the pan-glial network [106].

Similarly, the coumarin dipeptide, β-Ala-Lys-Nepsilon-Coumarin, specifically labels the rat brain-derived primary astrocytes in culture. However, it also stains oligodendrocyte precursors [107].

Moreover, it is also worth noting that fluorophores such as rhodamines, cyanines, and BODIPYs provide a unique opportunity to selectively and specifically induce strong fluorescence in the mouse and rat hippocampal astrocytes, without labeling neurons, as well as microglia precursor cells or oligodendrocytes [108].

ASTROCYTE-SPECIFIC GENETICALLY ENCODED MARKERS

It is known that astrocytes could be visualized in the cerebral slices by using cell-specific promoter-driven reporter fluorescent proteins selectively expressed in astroglia. For instance, the transgenic mouse strain was created showing the astrocyte-targeted expression of green fluorescent protein (GFP) or its analog enhanced green fluorescent protein (EGFP) driven by *GFAP* [109] or *S100β* [110] gene promoter thereby providing a valuable tool for thorough investigation of the dynamic change in the astrocytes during development as well as in pathophysiological states.

It is worth mentioning here that the number of astroglia-specific promoters has been progressively increasing so that by now a set of fluorescent protein probes with distinct spectral characteristics are available [111, 112]. In particular, a series of transgenic mouse strains expressing diverse reef coral fluorescent proteins (RCFP) displaying bright fluorescence covering a wide spectral range (from blue to red) driven by various gene promoters was generated [111].

Moreover, astrocytes could also visualized via expression of the genetically encoded Ca^{2+} indicators such as Yellow Cameleon-Nano 50 (YC-Nano50) [113] or Green GCaMP consisting of GFP fused to calmodulin and peptide sequence of myosin light chain kinase [114]. Moreover, some studies reported several GCaMP variants including GCaMP2, GCaMP3, GCaMP5, and red-emitting RCaMP tested in astrocytes [115, 116]. In particular, comparing GCaMP2 vs. GCaMP1 after inserting N-terminal 35-mer polyHis RSET (plasmid leader sequence) tag linked to calmodulin and myosin results in the enhanced brightness and thermostability [117]. However, a significant improvement was achieved by constructing GCaMP3 composed of the circularly permuted green fluorescent protein (cpGFP), calmodulin (CaM), and Ca^{2+}/CaM-binding myosin M13 peptide, which displayed increased brightness, wide dynamic range, and higher Ca^{2+} affinity allowing to report a surge in Ca^{2+} level triggered by single action potentials *in vitro* [118]. GCaMP5 was created based on GCaMP3 via site-directed mutagenesis and screening, which allowed to increase

dynamic range by severalfold compared to its predecessor GCaMP3 [116]. To generate a red-emitting RCaMP, cpEGFP was replaced with the red fluorescent protein (FP) mRuby in the GCaMP3 scaffold that allowed to increase thermodynamic stability [116].

Remarkably, astrocyte visualization *in vivo* with fluorescent markers could be also achieved by inoculating adeno-associated viral (AAV) and lentiviral vectors, administration of which could cause emergence of nuclear fluorescent signals specific to the diverse cell types allowing, in turn, to easily identify and distinguish cells of various origin. For instance, by using specific fluorescent labeling of neurons and astrocytes it allowed to distinguish these cell types spectrally due to expression of red fluorescent protein tdTomato in neurons and EGFP in astrocytes [119].

ASSESSING ASTROCYTES GENETIC PROFILE

Level of mRNA characterizes cell transcription state, therefore providing insight into the cell functionality, activity, and development, as well as magnitude of pathological remodeling upon disease progression. Human and mouse brain astrocyte transcriptomic profile were analyzed by using microtemplates and RNA sequencing combined with fluorescence-activated cell sorting (FACS) and cell panning of the astrocytes labeled by the transgene expression [120, 121].

In particular, assessing of the transcriptome in the cortical astroglial cells expressing green fluorescent protein (GFP) and freshly isolated from the adult transgenic FVB/N-Tg (GFAPGFP) 14Mes/J mice by using FACS allowed to demonstrate that the substantial proportion of the astrocyte-expressed genes (around 34%) were involved in cell metabolism [122]. Along with this, the subsequent PCR analysis revealed that the expression level for enzymes taking part in glycogen metabolism was higher in astrocytes than in neurons. While comparing astrocyte vs. neuron it was established that the relative expression of all glycolytic enzymes involved in the reactions starting from glucose phosphorylation to pyruvate synthesis was markedly upregulated in seven out of ten steps of glycolysis. Moreover, astrocytes also exhibited active oxidative metabolism manifested by the relatively high density of mitochondria, which suggested the opportunity for astrocytes to substantially contribute to functional brain visualization including blood-oxygen level-dependent functional magnetic resonance imaging signals [122].

In addition, it was shown using flow cytometry [33] that the S100β/EGFP-positive astrocytes expressing *S100β* promoter-driven EGFP were present in the S100β-EGFP transgenic mice aged 1-8 and 17-30 days. Comparison of the immature (age 1-8 days old) and mature (age 17-30 days old) astrocytes revealed that not be-

ing astrocyte-specific the genes highly expressed in the immature astrocytes are involved in cell proliferation and development (e.g., cell cycle genes), whereas those expressed in the mature astrocytes showed the astroglia-specific pattern. In particular, the latter include the genes encoding secretory proteins such as ApoE, ApoJ/clusterin, Pla2g7, Sparc, Sparcl1, and Mfge, some of which are also important for the astrocyte−synapse or astrocyte−endothelial cell interactions. Many of such genes are highly expressed in the 1-to-17-day-old astrocytes allowing to suggest that the relevant protein products play an important role in the CNS developing during formation of the majority of synapses and afterwards, which, in turn, is in agreement with the data on the ability of astrocytes to regulate synapse formation [123]. Moreover, astrocytes were also found to express other genes (*NPAS3*, *MLC1*, *LGI1/4*, and *GPR56*) participating in the development of mental diseases such as schizophrenia and bipolar disorder [124-126]. For instance, the *NPAS3* gene encoding transcription factor bHLH (basic helix-loop-helix) PAS (Per, Arnt, Sim) domain of the neuronal Protein 3 (NPAS3) is expressed in the Bergmann glia of the cerebellum [127]. The protein NPAS3 takes part in regulating neurogenesis, circadian rhythm, and cell proliferation. *NPAS3* gene mutations is considered as a risk factor for mental disorders [128]. The mice with *NPAS3* deletion demonstrate development of impaired neurogenesis and synaptic transmission in the hippocampus [129]. The *MLC1* gene encoding MLC1 membrane protein is specifically expressed in the distal astroglial processes in the perivascular, subependymal, and subpial areas of the brain. It consists of several transmembrane domains suggesting that it might exert a transport function [124]. The gene *LGI1/4* codes for the Leucine-Rich Glioma Inactivated Protein ¼, is expressed in astrocytes, and plays a crucial role in the synaptic transmission and myelination. For instance, *LGI4* takes part in regulating proliferation of glial lineage cells in the PNS and promotes Schwann cell myelination via binding to neuronal receptor ADAM22 [130]. The gene *GPR56* encodes the G protein-coupled receptor 56 (GPR56), which is expressed both in microglia, oligodendrocytes, and astrocytes, as well as in neuronal precursor cells and developing neurons [131, 132], participates in myelination, proliferation [133], and synaptogenesis [134].

The abovementioned facts allow to extend our understanding of the impact of astrocytes on synaptic regulation and development of mental diseases [135]. Indeed, based on the recently obtained data astrocytes may be directly involved in mediating synapse elimination in the developing and adult brain, which, in turn, plays an important role in remodeling of the brain synapse architecture [136].

The study assessing transcriptome profile of reactive astroglia in astrocytes isolated by flow cytometry from the adult transgenic Aldh1l1-EGFP mice after brain injury modeling seems to be very interesting [61]. It was discovered that the reactive astrocytes profoundly altered their transcriptional profile primarily affecting the genes encoding extracellular matrix proteins such as collagen (Col12a1, Col6a1) and versican (Vcan), thrombospondin (Thbs1) and fibulin 5 (Fbln5), CD44 and neurofascin (Nfasc). It suggests that the reactive astrocytes are able to modify extracellular matrix components during the glial scar formation. Likewise, the genes coding for intermediate filament proteins (GFAP, vimentin and nestin) were highly expressed in the reactive astrocytes, thereby reflecting morphological changes occurring upon activation. Moreover, a gene set encoding cytokines, constituents of the antigen presentation and the complement activation pathway were revealed, which were substantially upregulated in the reactive astrocytes suggesting that astrocytes may exert a regulatory activity upon interaction with immune cells during brain injury [61].

It is important to note that comparing transcriptome profiles among several astrocyte populations expressing specific cell markers, e.g., GFAP- vs. GLT-1-positive astrocytes [122], ALDH1L1- vs. GLT-1-positive astrocytes [32], and ALDH1L1- vs. GlialCAM-positive astrocytes [137] revealed no prominent differences between them suggesting that the majority of astrocyte populations co-expressed such markers.

A single-cell transcriptomic analysis represents one of the tools for determining astrocyte molecular heterogeneity. For instance, several studies investigated the single-cell transcriptomics profile of astrocytes [47, 138], which, however, were not continued to further assess astrocyte heterogeneity. However, a large-scale single-cell RNA sequencing for mouse cortical and hippocampal CA1 cells allowed to identify two astrocyte subsets based on GFAP and Mfge8 expression. In particular, the astrocytes expressing GFAP comprised a glial limiting membrane − the upper layer of the cerebral tissue located immediately beneath the pial membrane, whereas another subset of astrocyte was located more homogenously in the cortex and characterized by less branched processes [47].

OPTOGENETIC ASTROCYTE STIMULATION

Optogenetics represents a valuable tool for investigating astrocyte reaction to transduction of signals specific to the diverse types of neurons based on using opsins as light-sensitive proteins, which include microbial ion channels and ion pumps as well as engineered GPCRs [139]. Opsins absorb certain wavelength of incoming light causing a conformational shift that triggers diverse changes in the opsin-expressing cells. In the process, some opsins [channelrhodopsin-2 (ChR2), halorhodopsin, archerhodopsin (Arch), archerhodopsin-T (Arch-T)] induce ion translocation [140-142], whereas others (melanopsin) activate intracellular cascades such as G-protein signaling pathways [143].

Importantly, optogenetics allows to selectively target certain cell populations particularly astrocytes to be specifically acted upon, which, in turn, allows to conduct deeper and more detailed analysis of their functioning in the CNS [8]. Some studies demonstrated that optogenetic astrocyte stimulation via expression of light-sensitive ion channels and receptors causes significant changes of their functional plasticity [144, 145].

It was found out that stimulation of Opto-a1AR (a metabotropic opsin) expressed in the mouse hippocampal astrocytes could enhance the long-term synaptic plasticity, while the cation membrane channel ChR2 (ionotropic opsin) did not exhibit the so profound effect. It demonstrates that the long-term synaptic plasticity could be modulated optogenetically, which could be used to normalize synaptic transmission and correct impaired plasticity in some neurodegenerative diseases such as Alzheimer's disease [146].

It was shown that the optically activated astrocyte ChR2 could stimulate ATP release which, in turn, enhanced synaptic excitatory currents via stimulation of P2Y1 and A2A receptors on astrocytes [147]. However, the recent study using the newly designed approach based on the genetically encoded Ca^{2+} indicator GCaMP6f along with expression of the selective astrocyte melanopsin (G-protein coupled photopigment) to trigger Ca^{2+} signaling demonstrated that melanopsin was able to stimulate inositol-1,4,5-triphosphate (IP3)-dependent Ca^{2+} signal transduction and cause the ATP-dependent transient boost of hippocampal excitatory synaptic transmission via purinergic activation of P2Y1 and A2A receptors. Upon that, in contrast to melanopsin, the astrocyte Ca^{2+} signals induced by ChR2 stimulation were observed after longer (>5 s) light pulses. Moreover, low frequency light stimulation of the melanopsin-transfected astrocytes could cause long-term potentiation that activates episodic-like memory, thereby making melanopsin an optical tool for regulating a wide range of regulatory actions of astrocytes in the neuronal networks *in vivo* [143].

Another study with the adult (2-5-month-old) tTA-MlC1-tetO-ChR2(C128S)-EYFP mice (developed by Tanaka et al. [148]) investigated sustained Ca^{2+} response in the optogenetically targeted neocortical astrocytes. In this model, transgenic animals express a highly light-sensitive channelrhodopsin-2 mutant ChR2 (C128S) in astrocytes (under the *MlC1* gene promoter), which becomes activated and deactivated with blue-light and yellow-light pulses, therefore allowing to combine optogenetic ChR2 stimulation along with visualized astrocyte Ca^{2+} activity by using the Ca^{2+} indicator Rhod-2 AM (red spectral range). Hence, it provided an opportunity to assess astrocyte activation/excitability and determine the role played by astrocytes in pathogenesis of some cerebral diseases such as epilepsy, Alzheimer's disease, and Parkinson's disease [149].

Moreover, examining the effects of stimulated astrocytes on input processing in the layer 5 pyramidal neurons (L5PN) revealed that the optogenetically activated astrocytes in the vicinity of L5PN cell body prolong their firing. The observed effects were related to the release of S100β, an astrocytic Ca^{2+}-binding protein decreasing Ca^{2+} release into extracellular space. Upon that, extracellular Ca^{2+} level causes L5PN firing mediated by activation of Nav1.6 channels [150].

Interestingly, it was recently discovered that excitotoxicity in the Bergmann glia due to chronic optogenetic ChR2 activation results in the decreased glutamate uptake revealed by increased excitatory postsynaptic currents in Purkinje cells, which is in agreement with the verified downregulated EAAT-1 glutamate transporter expression [151].

Furthermore, physiological role played by astrocytes *in vivo* was examined in the transgenic Optoα1AR TG mice with astrocytes expressing the optogenetically activated Gq-GPCR Optoα1AR, photostimulation of which ensures a transient rise in the astrocytic Ca^{2+} level detected by Rhod-2 AM [152]. It allowed to assess the effects of modulated astrocyte stimulation on neuronal activity mediated via the astrocyte Gq signaling. In particular, the optogenetically activated Gq signaling in cortical astrocytes was found to transiently suppress neuronal activity via adenosine A1 receptor. Moreover, sensory stimulation along with the astrocyte activation induced a prolonged depression of the sensory-evoked response. Behavioral testing revealed that the astrocytic activation resulted in the long-term memory enhancement (a novel object recognition task) without affecting the short-term memory (Y-maze) [152].

Considering that the light-induced activation of the astrocyte expressing ChR2 alters the dynamic baseline Ca^{2+} level [153], mathematical modeling could be a great tool to predict how biophysical properties of the ChR2 construct as well as specifics of laser irradiation may affect astrocyte Ca^{2+} signaling. In this regard, creating a mathematical model would provide an opportunity to optimize the light stimulation regime to achieve anticipated Ca^{2+} level in astrocytes as well as to engineer future application-oriented optogenetic variants aimed at comprehensive investigation of the brain astroglial cells [154].

ASTROCYTE PHENOTYPIC ALTERATION IN PATHOPHYSIOLOGY

As a result of negative stimuli contributing to neuronal injury, astrocytes undergo some phenotypic and functional changes accompanied by development of reactive astrogliosis. Upon that, hypertrophy and upregulated GFAP expression are observed that facilitate formation of the typical reactive astrocyte phenotype, which in turn, proliferate, migrate, and are transformed

into the scar-forming astrocytes [155]. Similar to the reactive astrocytes, the scar-forming astrocytes demonstrate high expression of a set of protein markers such as GFAP, β-catenin-1, nestin, and N-cadherin. However, there are also gene markers specific for reactive astrocytes − matrix metalloproteinase-2 (*MMP-2*, encodes MMP-2 protein), *PLAUR* [soluble urokinase plasminogen activator receptor (suPAR)], matrix metalloproteinase-13 (*MMP-13*, encodes collagenase 3 protein), *Axin-2* (axin-like protein), a gene encoding an intermediate filament protein − nectin, and *CTNNB1* (protein catenin β-1), while gene-markers of the scar-forming astrocytes include *Cdh2* (N-cadherin), *SOX9* (SRY-Box Transcription Factor 9) as well as the chondroitin sulfate proteoglycans-related genes − *CSGALNACT1* (chondroitin sulfate N-acetylgalactosaminyltransferase 1), *CHST11* (carbohydrate sulfotransferase 11), *ACAN* (aggrecan), and *SLIT2* (Slit Guidance Ligand 2) [155]. Hence, along with the astrocyte morphological characteristics these gene markers may be used for reactive astrocyte and astrocyte phenotyping.

Two types of phenotypes are recognized in the reactive astrocytes − A1 and A2. While the type A1 reactive astrocytes produce pro-inflammatory cytokines able to cause neuronal death, the type A2 astrocytes activate neurotrophic factors contributing to neuronal survival and growth [156]. It must be noted that such reactive astrocyte phenotypes could be identified according to the specific genetic traits. For instance, the post-mortem examination of brain tissues from the patients with Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis revealed that the complement component 3 (C3) was at peak expression in the A1 astrocytes. At the same time, *S100A10* was found to be solely expressed in the type A2 astrocytes in the post-mortem brain tissues from multiple sclerosis patients [157]. Hence, it provides an opportunity to use such genes as markers specific to A1 and A2 astrocytes, respectively.

Activated microglia causes transformation of astrocytes to type A1 astrocytes by the released cytokines IL-1α, TNF, and C1q, each of which is important for inducing A1 astrocyte phenotype [157]. In the process, Mfge8 regulates astrocytic A1/A2 conversion by activating PI3K/Akt-axis and suppressing NF-κB-pathway.

In contrast, the type A2 astrocytes exert a neuroprotective effect on nervous system by secreting neurotrophic factors. Expression of the *S100a10* gene in A2 astrocytes is necessary for cell proliferation, membrane repair, and inhibition of cell apoptosis [157]. Moreover, the type A2 astrocytes promote expression of the pro-inflammatory cytokine − transforming growth factor-β (TGFβ), involved in synaptogenesis and exerting a neuroprotective activity.

The data from one of the studies investigating several phenotypes of the activated astrocytes and regulatory mechanisms involved are worth mentioning [158]. It was found in this study that the activated astrocytes *in vitro* and *in vivo* displayed two distinct functional phenotypes in regard to expression of pro- or anti-inflammatory genes, glial fibrillar acidic protein, as well as neurotoxic or neuroprotective activity. Moreover, it was also demonstrated that the activated astrocytes released protein lipocalin-2 (LCN2) regulating both astrocyte morphological transformation related to reactive astrogliosis as well as migration and apoptosis. Moreover, LCN2 contributed to the pro-inflammatory astrocyte activation, which, however, occurred simultaneously with the inhibition of IL-4/STAT6-axis signaling by participating in the alternative anti-inflammatory activation. Thus, it allows to assume that the astrocyte LCN2 secretory protein acts as an autocrine modulator in astrocyte functional polarization in the developing neuroinflammation that might be aimed at suppressing pro-inflammatory astrocyte activation as well as related neurodegenerative diseases [158, 159].

ASTROCYTES miRNAs AS POTENTIAL TARGETS FOR NEURODEGENERATIVE DISEASE THERAPY

MicroRNAs are 21-24 nucleotide-long small regulatory RNAs involved in post-transcriptional gene regulation via binding to messenger RNAs (mRNAs) based on complementarity sequence, which results in degradation or suppressed translation of the latter [160]. Moreover, microRNAs possess a potential to control hundreds of diverse transcripts [161]. One of the crucial features related to microRNA is its ability to be transported between the cells that allows microRNAs to exhibit a broad influence at tissue and organismal level [162].

It is known that virtually all cells secrete exosomes [163]. In particular, the recent study [164] investigating molecular profile of astrocytes and neuronal exosomes released into the extracellular space and ensuring intercellular communication via transfer of diverse cues (proteins, RNAs, lipids) showed that only some cellular microRNAs were selectively incorporated into exosomes both in astrocytes and neurons. Furthermore, the existing difference in the microRNA profiles between the exosome and the relevant cell type implies that the process of selective microRNA loading into exosomes is tightly regulated and may show a cell type-specificity.

Both acute and chronic CNS lesions cause reactive, structural, and functional changes in astrocytes defined as reactive astrogliosis. Impaired astrocyte functioning may substantially contribute to the progression of pathological process due to activated excitotoxicity, oxidative stress, and inflammatory responses [165]. Neurodegenerative diseases, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, in particular, are characterized by neuroinflammation, cognitive and

motor dysfunction, which is related to irreversible neuronal degeneration and apoptosis in the brain and spinal cord. However, it has been increasingly evident that the experimental data demonstrate a crucial role played by astrocytes in pathogenesis of chronic neurodegenerative diseases [166].

Impaired regulation of gene expression is a typical trait both during physiological senescence and neurodegenerative diseases [167]. Some studies suggest that microRNAs are involved in pathogenesis of neurodegenerative diseases by regulating astrocyte functions via diverse mechanisms, thereby able to act as potential therapeutic targets. For instance, the mice with the genetically modelled Alzheimer's disease (3xTg-AD strain) were shown to have elevated level of miR-181a that suppresses expression of certain synthetic proteins in astrocytes, GLT-1 in particular, enabling regulation of synaptic plasticity [168]. Moreover, miR-155 and c-Jun were observed to become activated during the amyloid-beta-induced increase in microglia and astrocyte activation that contributed to production of inflammatory cues such as IL-6 (interleukin-6) and IFN-β (interferon-beta) [169].

It was shown that the mouse model of Alzheimer's disease (APPswe/PS1/E9 strain) was associated with activated astroglial CEBPD (CCAAT Enhancer Binding Protein Delta − a key transcription factor during cerebral inflammation) that resulted in the altered spatial learning and memory due to activated miR-135a that inhibits activity of the neurotropic factor Thbs1 (thrombospondin 1), which, in turn, suppresses synaptogenesis as well as reduces dendrite growth and axonal sprouting [170].

Parkinson's disease is associated with diverse pathological factors including α-synuclein toxicity and other mechanisms. It was discovered that the serum levels of miR-153 and miR-223 decreased progressively in the wild type (WT) mice and the genetically engineered Parkinson's disease model mice (GFAP.HMOX1 strain) [171]. Along with that, the circulatory level of both microRNAs was lower in the transgenic vs. control WT mice, whereas α-synuclein level was elevated in the GFAP.HMOX1 mice vs. the WT mice. Hence, it allowed to suggest that miR-153 and miR-223 negatively regulate α-synuclein in the basal ganglia of the GFAP.HMOX1 mice [171].

On the other hand, it is also worth noting the study [172] assessing survival of the motor neuron exposed to extracellular vesicles secreted by the astrocytes isolated from the patients with amyotrophic lateral sclerosis. It was found that the extracellular vesicles could not only induce death of the motor neurons, but also contained microRNAs that negatively regulate gene expression particularly showing that the miR-494-3p suppresses expression of the genes including the one encoding semaphorin 3A (SEMA3A), which is involved in axonal growth and maintenance.

Noteworthy is the fact that the microRNA isolated from the motor neurons damaged during development of amyotrophic lateral sclerosis could also exert direct negative effect on the structural and functional integrity of astrocytes, which intensified neuronal degeneration significantly [173]. For example, the *in vitro* experiments data showed that astrocytes were able to take up neuronal miR-218, which, in turn, inhibited activity of the astrocyte glutamate transporter EAAT-2 due to direct impact on 3'-UTR EAAT-2 bearing several sites for miR-218 binding. However, blocking of the miR-218 caused significant increase of the EAAT-2 level in the mouse astrocytes in the amyotrophic lateral sclerosis model, thereby posing it as a promising therapeutic target. Thus, astrocyte dysfunction and reactive astrogliosis occurring due to the motor neuron injury in the developing amyotrophic lateral sclerosis could be mediated by the increased activity of microRNAs released from the damaged neurons [173].

Taken together, both *in vivo* and *in vitro* data indicate that microRNAs substantially impact pathogenesis of neurodegenerative diseases by directly or indirectly regulating astrocyte functions, which is manifested by activation of pro-inflammatory cytokines, suppressed synaptogenesis, neuron death, and mitochondrial dysfunction.

CONCLUSIONS

Astrocytes are crucial regulators in the developing and homeostatic brain as well as in emerging and progressing neurodegenerative diseases. Heterogeneity of astrocytes manifested by the diverse brain location-specific morphological traits is an important feature, which could complicate their further investigation. In connection with this, a detailed study of astrocyte gene expression patterns and their functional features in different brain areas may be of paramount importance. Unfortunately, this issue has not been fully resolved yet due to the lack of a universal marker for labeling astroglial cells typically observed in various brain regions. Moreover, none of the ideal astroglia-specific promoter ensuring reporter gene expression solely in this type of cells has been identified yet.

Hence, investigating molecular mechanisms underlying astrocyte−neuron intercellular interaction as well as discovery of novel functions of astroglial cells facilitates the search of new markers of activated astrocytes as well as approaches for their visualization. Combining visualization techniques with modern assays (PCR analysis, electrophysiology, optogenetics) would allow to obtain deeper insights into the multifaceted astrocyte functions, which could be crucial for understanding biology of the central nervous system astroglia in health and disease.

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