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Review Article

The critical role of astrogenesis and neurodevelopment in Fragile X Syndrome and Rett Syndrome

Zhuo Jun Li[a] and John-Paul Oliveria[a,b*]

a McMaster University

b Stanford University

Abstract

Astrocytes play an important role in the development of functional neural circuits in the brain; they are responsible for coordinating synapse formation and function, axon guidance, and ensuring neuronal survival. A better understanding of these mechanisms and their timing will further characterize its role in neurodevelopmental diseases. This paper will discuss the proposed pathways of astrogenesis, and genetic mutations that give rise to Fragile X Syndrome (FXS) and Rett Syndrome (RS). Normal astrogenesis begins during late gestation and is regulated by both cell intrinsic and extrinsic pathways. However, disruption to astrogenesis have been linked to abnormal astrocyte development and results in pathologies such as FXS and RS. FXS and RS are a result of genetic mutations that inhibit astrocyte function in FXS, and disproportionately activate astrocyte function in RS. Both FXS and RS have been associated with the theory that altered gene transcription during neurodevelopment disrupts astrogenesis, and subsequently, the behavior and function of mature astrocytes in the brain. Overall, current research has focused on the impact of the genetic mutation on the developmental pathway of astrocytes, and how the subsequent changed astrocytes play a role in the pethogenesis of FXS and RS. The existing gaps in knowledge around the timing of initial astrogenesis and identifying astrocyte-specific markers indicates more research is needed to discover the extent astrogenesis are affected by genetic mutations, and how scientists can improve upon existing techniques in studying astrocytes.

Keywords: astrocyte; astroglia; astrogenesis; neurodevelopment; Fragile X Syndrome; Rett Syndrome

*Corresponding author: oliveria@standford.edu

List of Abbreviations

Abbreviation	Definition
bFGF	basic fibroblast growth factor
BMP	bone morphogenetic protein
CNS	central nervous system
CT-1	cardiotrophin-1
EAAT-1/GLAST	excitatory amino acid transporter 1/glutamate aspartate transporter 1
EAAT-2/GLT-1	excitatory amino acid transporter 1/glutamate transporter 1
EGF	epidermal growth factor
FMR1	Fragile X mental retardation gene 1
FMRP	Fragile X mental retardation protein
FXS	fragile X syndrome
GFAP	glial fibrillary acidic protein
gp130	glycoprotein 130
HLH	helix-loop-helix
IL-6	interleukin-6
JAK-STAT	Janus Kinase/Signal Transducer and Activator of Transcription
LIF	leukemia inhibitory factor
MeCP2	methyl-CpG-binding protein 2
NFIA	nuclear factor 1 A-type
NSC	neural stem cell
NT-3	neurotrophin-3
RG	radial glia
STAT3	signal transducer and activator of transcription 3
TNC	Tenascin C

Introduction

The central nervous system (CNS) consists of neurons and glia. The latter is also subdivided into astrocytes and oligodendrocytes. Astrocytes, the most abundant non-neuronal cells of the CNS, are known as the choreographers of the neural circuit[1]. They play a critical role in developing the functional neural circuits of the brain, coordinating synapse formation and function, ensuring neuronal survival, and axon guidance[2,3]. As new neurons form in the developing brain, the brain is subsequently populated by astrocytes, which contribute to the formation and maintenance of neural circuits in the early postnatal brain by controlling synapse formation, function, and elimination[2]. In the mature brain, astrocytes perform multiple roles like producing and recycling neurotransmitters, regulating extracellular ion concentrations, and providing structural support to neurons and the blood brain barrier[2,4].

Neurons, astrocytes, and oligodendrocytes are differentiated from a common neuroepithelial origin in a temporally defined manner[2]. First divisions of neural stem cells (NSC) are thought to be exclusively neurogenic in early gestation and primarily gliogenic in late gestation [5,6]. Astrocyte development is proposed to begin with NSCs that differentiate into astrocyte precursors[5]. Local proliferation then allows these precursors to mature into astrocytes (Figure 1), which are divided into two major subtypes: fibrous and protoplasmic. Fibrous astrocytes, also known as white matter astrocytes, have fewer but thicker processes and typically express higher levels of the astrocyte intermediate filament glial fibrillary acidic protein (GFAP)[6,7]. They typically have regular cylindrical processes and contours, forming the more classic star-like appearance[7]. Their diverse range of function includes regulating the flow of blood through the CNS, maintaining synapses, and storing and releasing nutrients[7]. In grey matter, protoplasmic astrocytes have elaborate processes and eventually arrange into spatially segregated astrocyte domains of adult brains[6,7,8]. Protoplasmic astrocytes contain hundreds of fine processes, with the smallest endfeet directly contacting neuronal synapses, creating tripartite synapses[8]. These astrocytes participate in synaptic communication in a variety of direct and indirect functional pathways, such as clearing glutamate, modulating synapse functions, and regulating local capillary blood flow[8]. Each astrocyte cell forms a well-defined non-overlapping territory.

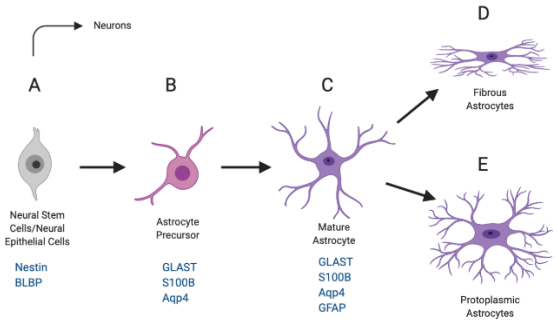

Figure 1 - Process of Astrocyte Development and Maturation. (A) Astrocytes begin as a neural stem cell during early pregnancy, and express predominantly markers for neurogenesis, such as Nestin and brain lipid-binding protein (BLBP). (B) During late pregnancy (in the fetal phase), stem cells begin to express astrocyte-related markers such as GLAST, S100B, and Aqp4, which allows them to become astrocyte precursors. (C) Through local proliferation, astrocyte precursors become functionally mature astrocytes, and now express additional astrocyte-specific markers (ex. GFAP). The mature astrocytes can specify into either (D) fibrous astrocytes or (E) protoplasmic astrocytes depending on their location within the central nervous system.

As mentioned previously, astrogenesis occurs during late-gestation and early postnatal stages. Astrocytes are generated from NSCs through three main proposed pathways: Notch signaling, BMP signaling pathway, and IL-6, in collaboration with the JAK-STAT pathway[9,10]. Astrogenesis is regulated by both cell intrinsic programs and cell extrinsic cues; disruption within the pathways result in abnormal astrocyte differentiation and function. Both Fragile X-Syndrome (FSX) and Rett Syndrome (RS) are rooted in genetic mutations that lead to astrocyte dysfunction.

Time (In Chronological Order)	**Stages of Astrogenesis**
1. Embryonic Phase/Early Pregnancy	I. RGs are derived from the lateral wall of the neural tube, and migrate radially along the cerebral wall
	II. Methylated astrocyte-specific genes
	III. Neurogenesis occurs
2. Fetal Phase/Late Pregnancy	I. RGs accelerate the expansion of the neuronal population, and subsequently switch to gliogenesis to produce astrocytes
	II. Astrocyte precursor cells migrate throughout the CNS
	III. Chromatin remodeling through demethylation of astrocyte-specific promoters begin astrogenesis and silence neurogenesis
	IV. Exogenously secreted cues (ex. IL-6, BMP family signaling, and Notch signaling) initiates and maintains astrogenesis
3. Postnatal Phase	I. Differentiated astrocytes begin to display diversity in morphology
	II. Astrocyte processes extend and astrocyte domains become recognizable
	III. Astrocytes matures into terminally differentiated state, but functional identity is not completely hardwired.

Table 1 – Summary of Astrogenesis. The stages of astrogenesis through early pregnancy, late pregnancy, and postnatally, including the breakdown of the stages of astrocyte development during each time period.

Initiation of astrogenesis: intrinsic chromatin remodeling

Astrocyte differentiation from NSCs is a temporally regulated phenomenon that relies on exogenously secreted cues and intrinsic chromatin changes[2,11,12]. Notch signaling is the master regulator of astrocyte differentiation[13,14]. This pathway represses neurogenesis when the fetus reaches late-gestation, and induces astrocytic differentiation through intrinsic chromatin remodeling[10,13,14]. Until late gestation, NSCs are insensitive to cytokines related to astrogenesis, like IL-6, because they have not obtained astrogenic potential[11,12,15]. The downstream GFAP gene is only activated in neuroepithelial cells in relatively late gestational stages, never in early or mid-gestation[9,15]. Acquisition of astrogenic potential is attributed to DNA demethylation at astrocyte-specific genes such as GFAP, S100β, and aquaporin 4[11,16]. Promoters of astrocyte-specific genes are highly methylated prior to late-gestation; CpG dinucleotides exist at the GFAP promoter region, impairing the activation of the GFAP gene and effectively silencing transcription[11,15,16]. When Notch signaling is activated, RBP-Jκ transcribes notch-target genes such as nuclear factor 1 A-type (NFIA)[15,16,17]. As a result, the increasing expression of NFIA induces demethylation of GFAP promoters, like the STAT3 binding site, and leads to GFAP expression in NSCs and signaling the beginning of astrogenesis[15,16,18]. Astrogenesis depends on STAT3 activation, as STAT3 is the transcription factor for the promoter region of the GFAP gene[15]. Exposure of the promoter allows NSCs to become responsive to external cues[16].

During the neurogenic phase, NSCs elongate to become radial glia (RG), and divide asymmetrically for auto-renewal and generation of neurons/neuron-restricted intermediate progenitor cells[19,20,21]. Newborn neurons migrate along parental RG fibers to their destination[20]. At late gestation, majority of RG cells have lost their ventricular attachment and migrate towards the cortical plate, subsequently dividing to become astrocytes[19,21]. During this gliogenic phase, RG progenitors gain competence to generate astrocytes due to the activity of growth factors like basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF)[19,22]. These factors allow RGs to respond to specific gliogenic signals acting at the extracellular level, and to respond to activated mature astrocyte markers like excitatory amino acid transporter 1 (EAAT1)/glutamate aspartate transporter 1 (GLAST), S100β, and aquaporin 4[19,23] (Figure 2). GLAST is a glutamate transporter that is functionally active in astrocytes, and its expression coincides with the gliogenic switch, making it a specific marker of astrocyte precursors in the spinal cord[23].

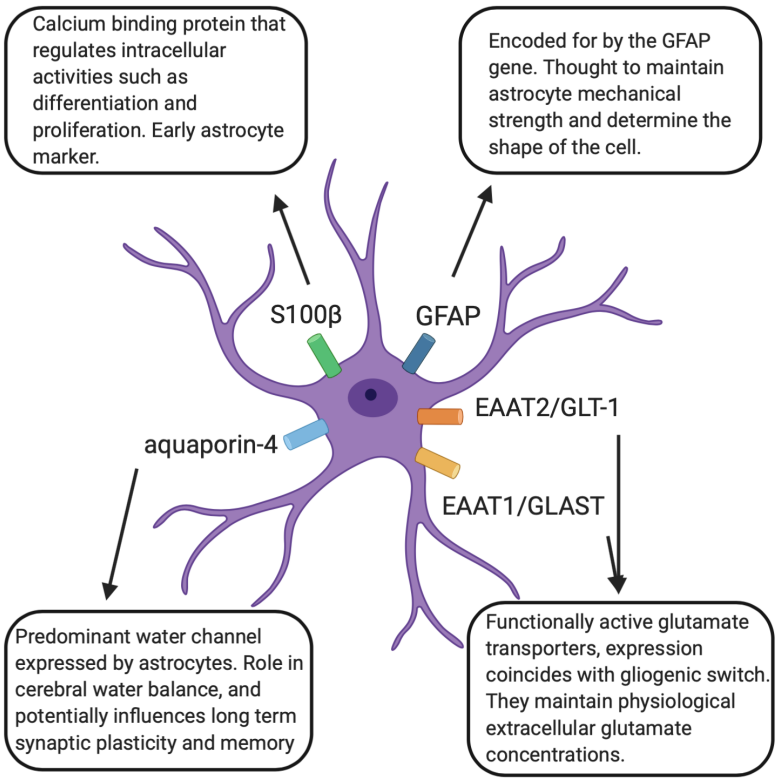

Figure 2 - Astrocyte marker expression profile. Five astrocyte-specific markers are often found on the surface of astrocytes, and aid in the role of astrocytes in the CNS. GFAP is thought to maintain astrocyte mechanical strength and determine the shape of the cell. S100B is a calcium binding protein that regulates intracellular activities. Aquaporin-4 is a water channel expressed by astrocytes, used to maintain cerebral water balance and influences synaptic plasticity and memory. EAAT1 and EAAT2 are both functionally active glutamate transporters whose expressions coincide with the gliogenic switch.

The synergistic effect of IL-6, BMP, and Notch signaling in promoting astrogenesis

There are three synergistic pro-astrogenesis pathways; IL-6 and cardiotrophin-1 (CT-1), BMP signaling, and Notch signaling. First, CT-1 binds to glycoprotein130 (gp130) and leukemia inhibitory factor (LIF) receptor beta coreceptors, which then signal via the JAKs to phosphorylate and activate the STAT3 transcription factors[10,13,15,24]. STAT3 then forms a complex with Smad (*Caenorhabditis elegans Sma* genes and the *Drosophila Mad,* Mother against decapentaplegic) proteins, which are downstream of BMP receptors. BMPs belong to a subset of transforming growth factor-B superfamily and includes multiple functional peptides that control proliferation and differentiation in various cell types, including astrocytes[10]. They also inhibit neuronal differentiation during late-gestation.

This pathway initiates when BMP2 and BMP4 bind to heterotrimeric serine/threonine kinase receptors to signal via the activation of downstream transcription factors Smad1, Smad 5, and Smad 8[10]. They form a transcriptional complex with the aforementioned activated STAT3 in the CT-1 induced pathway, which is then able to bind to astrogenic genes like GFAP[18]. At the same time, Notch, upon binding to its ligands, is activated and cleaved. The Notch intracellular domain then translocate to the nucleus where it interacts with RBP-Jκ to form a transcriptionally active complex that binds directly to the GFAP or S100β promoter and promotes transcription[24]. However, this must occur when the JAK-STAT pathway is also activated[18,25]. Notch1 mRNA is also upregulated during astrogenesis, suggesting that a feedback loop sustains Notch signaling until astrocytic differentiation is complete[19]. Together, these three pathways and a key proastrocytic transcription factor, NFIA, bind to the GFAP promoter (Figure 3). Their coordinated actions determine the timing and number of astrocytes that are ultimately formed[24].

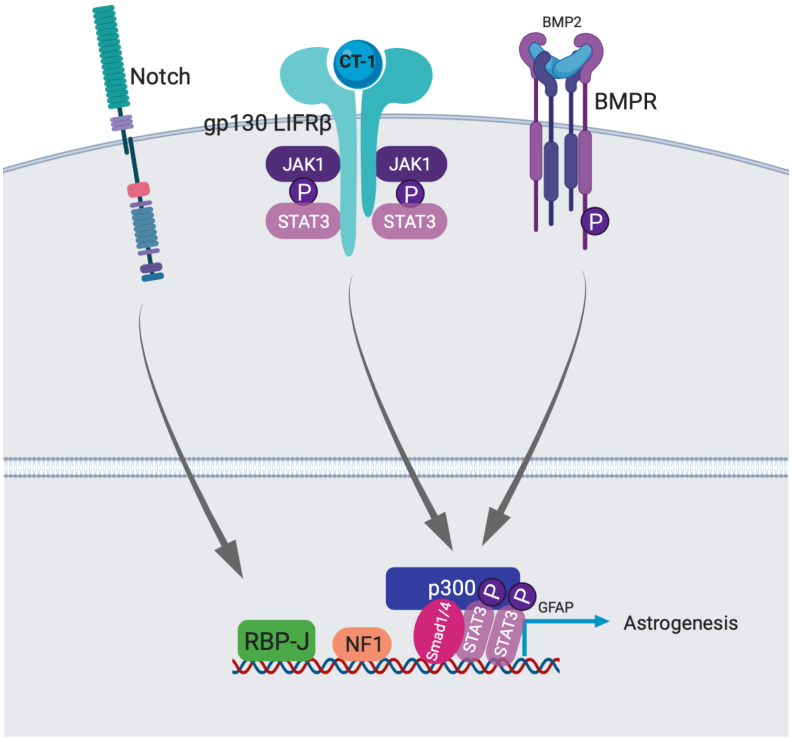

Figure 3 - Synergistic pathway of Notch signaling, BMP family signaling, and IL-6 signaling in conjunction with the activated JAK-STAT pathway to induce astrogenesis. First, CT-1 binds to gp130/LIFRB coreceptors, which induces downstream signaling via the JAK-STAT pathway molecules. Together, it forms a complex with downstream BMP signaling molecules. At the same time, Notch is activated and cleaved, and translocate to the nucleus to interact with RBP-J to form another transcriptionally active complex. Along with NF1, the two complexes bind to the GFAP promoter.

IL-6 and BMP signaling in promoting astrogenesis

IL-6 and BMP families synergistically induce astrocyte differentiation[9], and the JAK-STAT pathway mediates signal transmission into the nucleus[25]. In addition to CT-1, leukemia inhibitory factor (LIF) can also bind to leukemia inhibitory factor receptor (LIFR)/gp130 and forms a complex of LIF/LIFR/gp130[26]. In either situation, binding of an IL-6 family molecule (i.e., CT-1 or LIF) triggers JAK1 activation, which is constitutively associated with the cytoplasmic region of LIFR and gp130[26]. Subsequent protein to protein interaction and phosphorylation of the cytoplasmic region of gp130 and LIFR by JAK1 leads to recruitment and tyrosine phosphorylation of STAT3[9,18]. The dimerized STAT3 is translocated into the nucleus, where it binds to the promoter of the GFAP gene[18]. Other astrocyte inducers are the BMP family cytokines, such as BMP2. Similar to the pathway discussed above, BMP2 binds to a receptor complex composed of type I and II BMP2 receptors. Both receptors are membrane spanning serine-threonine kinases[9]. Upon binding of the ligands, type II receptor phosphorylates type I, which then goes on to phosphorylate downstream Smad proteins[9]. The activated ligands translocate to the nucleus together; this synergistic effect is required to induce astrocyte differentiation[25] (Figure 4). In the nucleus, STAT3 and Smad1 bind to their recognition sequences in the GFAP promoter[9,25]. Transcriptional coactivator p300 interacts with STAT3 at its amino terminus with Smad1 binding to its carboxyl terminus, leading to efficient expression of the GFAP gene[9]. This synergistic effect can be observed with any combination of the IL-6 family and BMP2, BMP4, or BMP7.

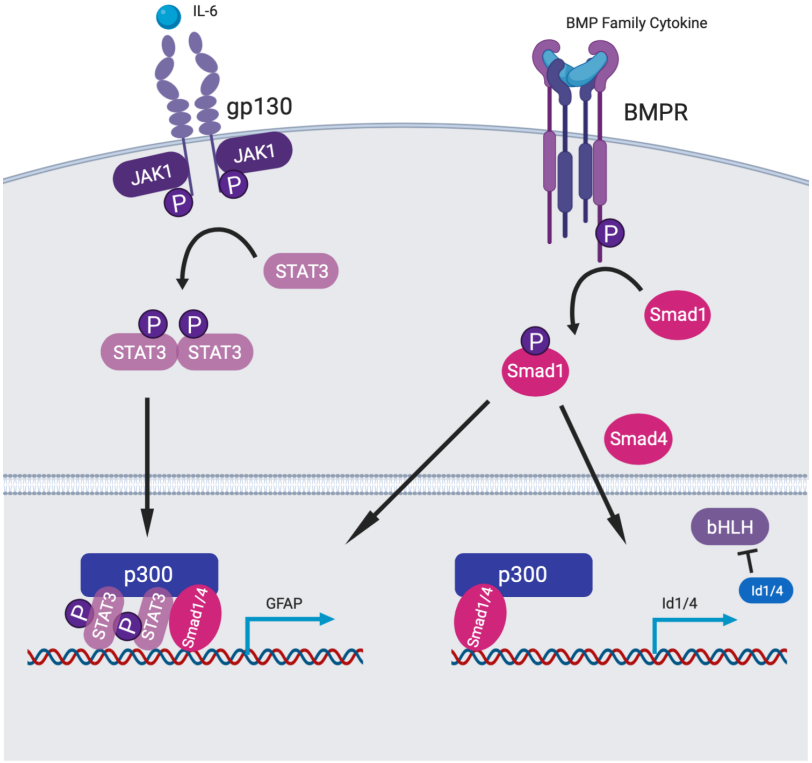

Figure 4 - Overview of the IL-6 BMP family signaling. IL-6 family molecules bind to the LIFR/gp130 coreceptor, triggering the activation of JAK1, leading to tyrosine phosphorylation and recruitment of STAT3. The dimerized STAT3 translocate to the nucleus, where they it binds to the GFAP promoter. At the same time, BMP2 binds to the BMP receptor complex, leading to the phosphorylation of downstream Smad proteins like Smad1, which then translocate to the nucleus. Downstream collaboration creates the P300/STAT3/Smad1/4 complex that binds to the GFAP gene to promote astrogenesis. BMP family molecules can also induce negative HLH factors that inhibit neurogenesis.

In addition to astrogenesis-activating pathways, BMP family molecules suppress neurogenesis to allow for the switch to astrogenesis. BMP2 alters the developmental pathway of NSCs from neurogenesis to astrogenesis by inducing negative helix-loop-helix (HLH) factors such as Id1, Id2, and transcription factor hes5[9,10,27] (Figure 4). These proteins inhibit the neurogenic bHLHs that normally promote neurogenesis and supress gliogenesis[13,16,27,28]. BMP2 is also shown to reduce the number of NSCs expressing a marker for undifferentiated neural precursors, like nestin, and increasing the number of cells expressing S100β**,** an early astrocyte marker[28]. This change initiates the switch from neurogenesis to astrogenesis. Polycomb repressive complex 2 (PRC2) also contributes to suppressing neurogenesis by catalyzing H3L27 methylation to silence the Neurogenin1 gene that codes for neurogenesis[28]. The silencing both terminates neurogenesis and stops Neurogenin1 from interfering with the P300-Smad1 complex of STAT3, which is essential for astrocyte development[16].

As seen from the multiple synergistic pathways and molecules involved with astrogenesis discussed above, astrogenesis is a tightly regulated process. Dysregulation at any point in this intricate dance can give rise to neurodevelopmental disorders such as FXS and RS

Abnormal Astrogenesis

Fragile X Syndrome

FXS is a neurodevelopmental disorder that affects 1 in 2500 individuals and is the most common cause of inherited brain development abnormalities[29]. Children with FXS mild to severe cognitive impairment, attention deficit, anxiety, susceptibility to seizures, motor disorders, and autistic behaviors[30,31]. FXS is linked to mutations in the fragile X mental retardation gene 1 (FMR1)[29,30], which codes for the fragile X mental retardation protein (FMRP). FMRP modulates the transcription of a number of mRNAs important for dendritic growth and development of the synapses[30], and is only expressed in the developmental stages of astrogenesis[29]. Individuals diagnosed with FXS have an abnormal number (>200) of CGG repeats in the 5' noncoding region of the FMR1 gene, resulting in hypermethylation and transcriptional silencing of the gene, meaning insufficient production of FMRP[29] (Figure 5). In absence of FMRP production, there is a disruption in the composition of normal protein milieu in astrocytes.

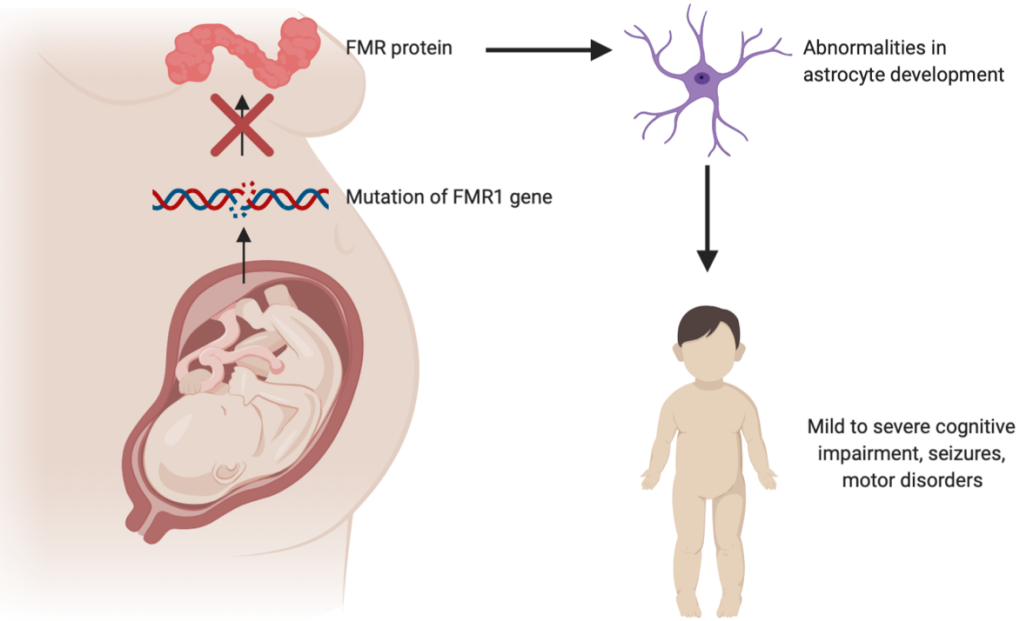

Figure 5 – Pathogenesis of Fragile X Syndrome. Mutation of the FMR1 gene during pregnancy causes insufficient production of FMRPs, which is a regulator of mRNA translation important for dendritic growth and synaptic development. Lack of FMRPs creates a disruption in the composition of normal protein milieu in cells like astrocytes, leading to abnormalities in astrocyte development. This leads to FXS, with symptoms such as cognitive impairment, seizures, and motor disorders.

Research assessing murine models of FXS show that astrocytes are involved in shaping the dendritic arbors of neurons in FXS[29]. Jacobs et al found that neurons exhibited a distinct abnormal morphology when grown with Fmr1 KO astrocytes, compared to neuronal growth with wild-type astrocytes[29]. This suggests a loss of fine-tuning in astrocyte-mediated neuronal growth, migration, and pruning in FXS. This results in complex dendritic arbors with increased branch density in neurons grown

with astrocytes from FXS mice, while expected levels of branch density was observed with normal astrocytes[29]. Astrocytes in FXS have also been found to be deficient in the ability to regulate synapse development, as neurons in FXS mice exhibit a decreased number of presynaptic and postsynaptic protein aggregates[32]. Thus, astrocytes could be a major contributor to erroneous synapse development and behavior maladaptation in FXS. The current theory on abnormal astrocyte development speculates that the reduction of FMRP could directly or indirectly contribute to abnormalities in astrocyte development[32]. In astrocytes, FMRP is expressed during development and its expression is downregulated as the brain matures[33]. It is possible that astrocytes lack FMRP specifically at a time during development when astrocyte support of neuronal growth and synapse formation are vital[29,32]. Because FMRP is a key regulator of translation, FMRP could potentially also regulates a subset of mRNAs in astrocytes[29]. Loss of FMRP would then result in abnormal protein translation in astrocytes, interfering with astrocyte-mediated neuronal growth and synaptic development, and contributing to the pathogenesis of the disorder[29]. Absence of FMRP in astrogenesis also contributes to the enhanced excitability of neurons in FXS. A study by Simhal et al shows Neurotrophin 3 (NT-3) as a possible mediator of this effect[34]. Release of NT-3 is elevated in astrocytes from FMR1 knockout (KO) mice, resulting in abnormal dendritic morphology and synaptic protein expression[34]. Loss of FMRP from astrocytes also leads to reduced expression of glutamate transporter GLT-1 (EAAT2) and subsequently reducing glutamate uptake by these cells, which contributes to neuronal hyperactivity and excitotoxicity[34]. Thus, astrocyte involvement in FXS pathogenesis is likely through impaired glutamate uptake. Another factor that may contribute to FXS pathogenesis is the astrocyte-secreted extracellular matrix glycoprotein Tenascin C (TNC)[35,36]. TNC is an endogenous ligand of toll-like receptor 4 (TLR4) that has been shown to induce the expression of pro-inflammatory cytokines such as IL-6[35,37]. ocytes from murine models, Krasovska et al found that secreted TNC and IL-6 were significantly increased, stimulating TLR4[35]. TLR4 activation may influence synaptic development, resulting in abnormal formation and maturation of excitatory synapses in FXS[35]. According to this body of literature, it can be concluded that astrocytes are impaired by the genetic mutation on the FMR1 gene, contributing to the pathogenesis of FXS. However, much is still to be learned about abnormal astrogenesis in FXS.

Rett Syndrome

RS is a X-linked neurodevelopmental disorder that affects around 1 in 12,000 girls[41]. Symptoms appear after 6-18 months of seemingly normal development[41]. These include seizures, cardiac and breathing problems, repetitive hand movements, and communication difficulties. The disease is predominantly explained by mutations of the methyl-CpG-binding protein 2 (MeCP2) gene, on the X chromosome[42,43]. During development, one X chromosome in each somatic cell is randomly inactivated, resulting in a blended expression of both mutant and healthy MeCP2 alleles[41,42]. Loss of MeCP2 occurs not only in neurons but also in glial cells, like astrocytes. This mutation is deadly in males, as they do not have another normal functioning MeCP2 gene[41]. MeCP2 normally binds to methylated portion of chromatin and can recruit factors to remodel the chromatin into an inactive state[41,42]. Specifically, MeCP2 inactivates astrocyte-specific genes so they would not be transcribed at an inappropriate time[41]. In normal neurogenesis, MeCP2 binds to methylated portions of astrocyte-specific gene promoters such as GFAP to silence transcription. As development progresses, methylation of the gene decreases and MeCP2 can no longer bind to the promoter and the chromatin remodels into an active state, allowing gene transcription

for astrogenesis[44]. Expression of MeCP2 in astrocytes is important for differentiation and function. Tightly regulated timing allows for the appropriate number of neurons and astrocytes to be generated during development[41,44]. In RS however, the switch to astrogenesis occurs prior to the appropriate time, as MeCP2 is mutated and cannot remodel the promoter's chromatin into an inactive state[41,42] (Figure 6).

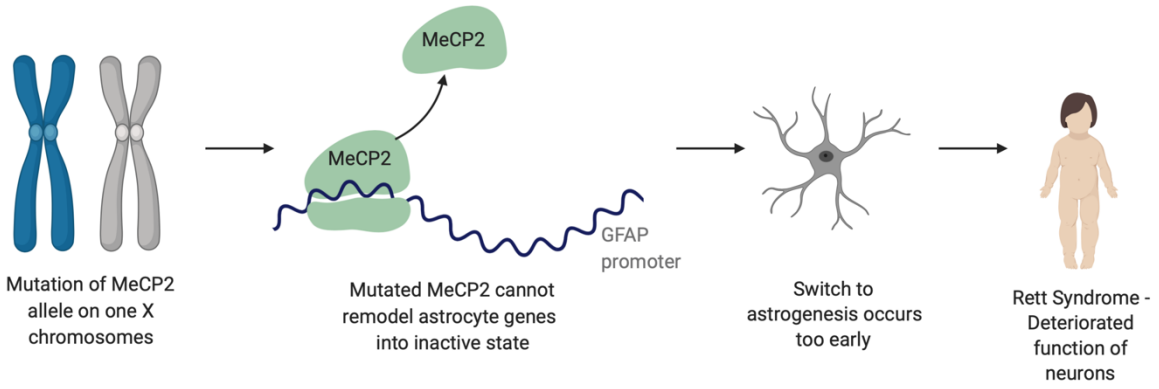

Figure 6 – Pathogenesis of Rett Syndrome. Mutation of the MeCP2 allele on one of the X chromosomes causes blended MeCP2 expression. Mutated MeCP2s are unable to remodel an astrocyte promoter's chromatin into an inactive state to allow for neurogenesis to occur during early pregnancy, thus the switch to astrogenesis begins too early. Incorrect neuron and astrocyte numbers being made results in RS, leading to cardiac and breathing problems, seizures, and communication difficulties in females who are affected.

Under normal conditions, healthy astrocytes promote dendritic growth when co-cultured with healthy neurons. However, MeCP2 +/1 mice and healthy neurons resulted in shorter dendrites and somas[43]. Astrocytes normally increase the frequency of both excitatory glutamatergic and excitatory GABAergic neuronal synaptic currents in a calcium dependent manner[45]. However, in MeCP2 null mice, astrocyte-mediated synaptic modulation is absent and calcium signals in astrocytes are severely blunted[45]. Growth rate of MeCP2-deficient astrocytes is significantly slower than normal, and more IL-1β and IL-6 are released[44,46]. They also have altered regulatory effects on neuronal morphology and function, due to certain abnormalities in target gene regulation and toxicity to neurons from abnormal glutamate metabolism[46,47]. This suggests that abnormal astrocytes are able to deteriorate the function of neurons.

GFAP and S100β expression are significantly higher in MeCP2-null astrocytes than in normal astrocytes[46,48] , suggesting that MeCP2 can couple with the Sin3A/HDAC complex to bind to the GFAP promoter and regulate its transcription[46,48]. Additionally, glutamate may induce both neuronal and glial death through excitotoxicity[46,49]. While extracellular glutamate concentrations are normally maintained by EAATs of astrocytes, astrogenesis dysregulation results in lower glutamate clearance rates [46,50]. Thus, the absence of MeCP2 expression during neurodevelopment has been linked to abnormal astrocyte function that may directly contribute to the pathological process of RS.

Overall, abnormal astrogenesis is shown to be induced by genetic mutations in both FXS and RS. FXS is attributed to mutations in the FMR1 gene, which codes for FMRP, an mRNA translation regulator that plays a crucial role in modulating transcription for dendritic growth and development of synapses[29]. RS is linked to a mutation in the MeCP2 gene, resulting in mutated MeCP2 that is unable silence transcription of astrocyte-specific promoters during neurogenesis[44]. Genetic mutations in both FXS and RS potentially results in deficient astrocytes that are unable to regulate synaptic development.

Future Directions

Understanding of the role of astrocytes in human neurological and psychiatric diseases requires a robust knowledge of astrogenesis and their role in neurodevelopment. Current research has shed much light on the factors regulating the gliogenic switch as well as the pathways to astrogenesis[1-28]. However, many questions are unanswered due to the difficult nature of astrocyte research. Astrocytes are difficult to study in *in vivo* systems because many of the key proteins and genes are expressed by multiple cell types[51]. It has been difficult to distinguish contributes of astrocytes from surrounding microglia because they reactive collectively, thus many of the pathways of astrogenesis contain missing pieces[51]. For example, scientists still lack detailed understanding of how the pathways simultaneously repress neurogenesis and promote gliogenesis[6]. Additionally, many early studies utilize only GFAP, a late astrocyte protein, as an indicator for astrogenesis due to a lack of appropriate markers[10]. However, GFAP expression doesn't distinguish astrogenesis from terminal astrocyte differentiation, and many neural precursors also express GFAP, making it not as exclusive to astrocytes as previous thought[10]. Limitations also exist in the research methodology used to study astrocytes and their pathways. Current models of signaling and pathogenesis are mostly based on murine models, and scientists are unsure as to how similar human and rodent astrocytes behave, especially in the context of complex disorders like autism[35].

Future research should be directed towards understanding the timing of the switch from neurogenesis to astrogenesis. Discovery of new markers of astrocyte maturation and diversity will also help to address heterogeneity in the brain. With regards to psychiatric diseases like FXS and RS, it is necessary to understand how astrocytes modulate synaptic development and function in neural circuits, especially with regards to their role in mediating cognition[34,35,45,46]. Progress on better characterizing the role of astrocytes in neurodevelopmental disorders require advancement in our understanding of the genetic factors contributing to neurological diseases and more sophisticated research methods and techniques[6]. Recent work has shown the feasibility of deriving functional astrocytes from embryonic stem cells. The in vitro differentiation process has been shown to follow in vivo developmental stages, where stem cell derived neuroepithelial cells transition from multipotent neural progenitors into more restricted astroglial progenitors over time[6]. This new process may allow an accessible human cellular system for better understanding the role of astrocytes within neurodevelopment in FXS and RS. Furthermore, using human induced pluripotent stem cell derived astrocytes from affected patients of FXS and RS could help to confirm the observations made in the rodent models and lead to potential therapeutic directions[2]. The stem cell cultures are advantageous because patterning molecules can be added during the neuroepithelial stage to specify progenitors, generating a wide variety of astrocyte subtypes to study[2]. It may provide functionally specific astrocytes for region-specific diseases and allow researchers to identify the molecular basis of the abnormalities that arise from neural diseases[6]. Considering our current understanding of the function and morphology of astrocytes, further research is needed to consolidate our knowledge of the role of astrocytes within the CNS, specifically with regards to the timing of astrogenesis, and the particular mechanisms by which astrocytes regulate neuronal development.

Conclusion

Astrocytes play an important role in the CNS. Besides common functions like modulating the neurovascular blood flow and regulating the extracellular ionic milieu, research has shown that astrocytes shape the synaptic environment and generate signaling mechanisms within neural networks. NSCs first begin to differentiate into neurons during early to mid-gestation, switching to gliogenesis in late gestation. This timing is tightly regulated by external cues and internal cell programming. First, chromatin remodeling occurs in astrocyte-specific genes, like GFAP and S100β. Then, through a combination of Notch, BMP and IL-6 signaling pathways in collaboration with the JAK-STAT pathway, NSC differentiation into astrocytes is promoted. However, genetic mutations that disrupt astrogenesis and subsequent astrocyte function facilitates the onset of neurodevelopmental disorders. FXS is implicated by a mutation in the FMR1 gene that results in the absence of FMRP, which is normally expressed in astrocytes. This interferes with astrocyte-mediated neuronal growth and synaptic development, contributing to the autistic symptoms characteristic of FXS. RS is also due to a genetic mutation in the MeCP2 gene, leading to loss of MeCP2 in astrocytes. Absence of MeCP2 contributes to various abnormalities in astrocyte function. Therefore, it is important to characterize the various stages of astrogenesis in order to understand the mechanisms behind neurodevelopmental disorders, and which pathways to target for therapeutic treatment.

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