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Abstract

The study of extracellular matrix (ECM) in the nervous system has longed been focused on the molecules promoting growth and migration. This is well supported by the work in the developing nervous system. However, the discovery of Nogo and chondroitin sulphate proteoglycans (CSPGs) in the injured nervous system in late 1980s has shifted some of the focus to inhibitory molecules. One of the biggest hurdles in neural regeneration is the formation of glial scar and the highly up-regulated inhibitory molecules present in the area. Apart from Nogo and CSPGs, other myelin-associated inhibitors, tenascins and semaphorins have been found associated with neuronal inhibition. Together with the identification of their receptors, we now have a better understanding on the mechanism of how these molecules control and limit regeneration in the central nervous system (CNS). Recent focus has been put on designing strategies in neutralizing these inhibitions for promoting regeneration after injury, and some are showing promising results. Moreover, latest studies also show that rehabilitation in injured animal models demonstrated drastic remodeling of ECM favoring regeneration. This review shall discuss all these different aspects and the importance of matrix remodeling in the CNS and the implication of ECM in some retinal pathologies.

Keywords: retina, regeneration, perineuronal nets, chondroitin sulfate, integrins

1. Introduction

The extracellular matrix (ECM) constitutes a three-dimensional network that surrounds the cells and conform the structure and characteristics to tissues. It has become increasingly evident that once being considered as a bystander between cells, the ECM indeed performs significant functions and involves in controlling various physiological responses in the CNS. The impor-
tance of ECM is at three levels: it acts as biological scaffold for the structure of the CNS and controls the diffusion and availability of molecules for biochemical signaling and communication and, finally, the various polymers and molecular interactions in the ECM control the biomechanical properties of the central nervous system (CNS) [1, 2]. In addition, regenerative capacity of tissues is also directly related to the ECM. Disorders in mechano-transduction or alterations in the composition of ECM will drive to a loss of the regenerative ability of the tissue and cells [3, 4]. Moreover, a proper immune and toxic response to infections is in accordance with the correct equilibrium in the ECM components [5].

In the nervous system, the ECM are synthesized and secreted by both neurons and glia. In the present chapter, we shall introduce the main key components of the ECM present in the brain and the main implications of these molecules associated to the normal and pathological CNS, including the spinal cord injury and in retina [6, 7]. While axon–glia interaction helps to determine the extent and direction of axon outgrowth, the growth of axons are also directed by factors present in the ECM. The growth enhancing cues such as laminin and fibronectin will encourage the growth and extension of neurites, while the inhibitory cues such as chondroitin sulfate proteoglycans (CSPGs) and semaphorins (Sema) serve as barriers in precise locations to prevent the growth of certain axon pathways into inappropriate areas.

2. ECM components

The ECM in the nervous system is mainly provided by macroglia and is an important source of supporting and signaling factors [8]. ECM components are key mediators of glial activation and have the capacity to evoke both regenerative and degenerative effects on glia and neurons [9]. The production of ECM components changes drastically during reactive gliosis [10]. The activated astrocytes and microglia increases the synthesis of various ECM molecules, including the re-expression of some extracellular glycoproteins, which are down-regulated after development [8, 10]. These include a complex mixture of proteins, proteoglycans (PGs), and glycoproteins (GPs) that confer the structural properties of cells and tissues.

2.1. Proteoglycans

Proteoglycans are macromolecules composed of a core protein on which multiple glycosaminoglycan (GAG) chains are attached (Figure 1). The GAG chains are linear unbranched polymers of repeating disaccharides composed of hexosamine and an uronic acid [11]. These molecules have remarkable physical properties attributable to the abundance of carboxyl, hydroxyl, and sulfate groups [11]. Their electrostatic properties make them “osmotically active”. Their net negative charge attracts Na+ and thus, draws water in causing the interstitial spaces where GAGs reside to swell. This swelling opens up pathways that promote the invasion and migration of cells as has been suggested for the non-sulfated GAG hyaluronan (HA), which is correlated with an initiation of cell migration during development [12]. There are five groups of GAGs based on the composition of the repeating disaccharides [11]. They include hyaluronan, chondroitin sulfate (CS), dermatan sulphate, heparan sulfate and keratan
sulfate. Except HA, all GAGs are covalently attached to a core protein and form proteoglycan (PG) (Figure 1).

Chondroitin sulfate (CS) and its proteoglycan CSPG constitute the major population of proteoglycans in the CNS [13, 14]. There are at least sixteen CS core proteins expressed in the CNS and many of them are produced by astrocytes [15]. The disaccharides in the CS chains can be sulfated at various positions resulting in different isoforms of CSs, including chondroitin 4-sulfate and chondroitin 6-sulfate, the most CS sulfation in an adult CNS (Figure 1) [16]. Together with the diversity of core proteins, the CS chain length, the number of chains attached to the core proteins, these factors give a huge diversity with a wide functional heterogeneity of CSPGs (Figure 1) [15].

CSPGs are known for their inhibitory influences on neurite extension [17, 18]. It was first demonstrated in dorsal root ganglion (DRG) neurons and subsequently being recognized in the CNS [18, 19]. CSPGs, such as NG2 and neurocan, are strongly up-regulated in the glial scar after injury [10]. Their up-regulation induces growth cone collapse and form a strong barrier for nerve regeneration [20]. CSPGs are primarily produced locally by the reactive astrocytes which are attracted to the peri-lesioned area after injury [9]. Chondroitinase ABC (ChABC), an enzyme which digests the CS chains into disaccharides, effectively removes this inhibition both in the developing CNS and after injury in adult [21–23]. ChABC removes the CSs in the developing hindbrain and promotes the neurite extension of commissural vestibular neurons in developing embryos [21]. Similarly, ChABC is very effective in removing the inhibition in the glial scar, encouraging sprouting and regrowth, and conferring functional recovery after
injury [22, 24]. Studies in recent years have been focusing on finding new strategies for sustained CS down-regulation, based on the success of ChABC, to provide a sufficient time window for regeneration to occur. The expression of ChABC using lentiviral vector show a down-regulation of CSs for up to 8 weeks, and the animal demonstrates an enhanced axonal sprouting and a superior functional recovery in the forelimb after a cervical contusion injury [25, 26].

One of the mechanisms of CSPGs mediating their functions is through binding to receptors or growth factors. Contactin-1, Nogo receptors (NgRs), and RPTPσ are the identified CSPG receptors [27–29]. The transmembrane receptor RPTPσ binds to CSPGs neurocan and aggrecan in the CNS [28]. RPTPσ induces growth cone collapse in vitro [30]. Mice with RPTPσ knockout show a robust regeneration, with cortical spinal tract fibers extend for a long distance after a spinal hemisection [31] Blocking the binding of CSPGs to RPTPσ using membrane-permeable peptide mimetics promotes serotonergic innervation, and the animal demonstrates enhanced functional recovery after spinal cord injury [30, 31]. This membrane-permeable peptide mimetics hold a strong promise to modulate CSPG-mediated inhibition in replacing ChABC. Apart from RPTPσ, NgRs also bind to CSs. Double mutant of Ngr1/Ngr3 showed an enhanced axon regeneration in optic nerve crush injury [29]. Contactin-1 binds specifically to highly-sulfated CS, CS-E, on the contrary to other CSPG receptor, the binding of CS-E to contactin-1 promotes neurite extension [27]. Neuro2a cells with recombinant expression of contactin-1 demonstrate extensive neurite sprouting when cultured on CS-E [27]. CSPGs also interact with other growth factors, chemokines, and guidance molecules in the developing brain. By doing so, they control the availability of these factors to cells. While fibroblast growth factor (FGF)-2, FGF-10, FGF-16, and FGF-18 bind to highly sulfated CS chains, guidance proteins such as slit2, netrin1, ephrin A1 and A5, semaphorin (Sema) 3A, 5A, and 5B bind to CS chains in a sulfation-dependent manner [32].

CSPGs in the CNS also aggregate into a macromolecular structure on the surface of neurons called perineuronal nets (PNNs) [33, 34]. PNNs are dense matrix structures formed by four families of brain ECM molecules, including CSPGs, hyaluronan, hyaluronan, and proteoglycan link proteins (HAPLNs) and tenascins [33]. PNNs wrap the neuronal surface and are crucial in controlling synaptic and neuronal plasticity in the developing and injury CNS [4, 35]. PNNs form toward the end of the critical period for plasticity, and the formation is activity dependent [36, 37]. Dark rearing from birth delays the formation of PNNs in the visual cortex [36]. Similar observation is also reported with whisker trimming from birth in the barrel cortex of mice [38]. ChABC treatment removes this layer of CSPG-enriched PNN matrix and reacti-
vates plasticity in the adult CNS, this includes spinal cord injury, visual cortex plasticity, cuneate nucleus plasticity, and more recently, on memory enhancement [35, 39, 40]. PNNs mediate their functions, in part, through binding to other molecules such as chemo-repulsive molecule sema3A and soluble transcription factor Otx2 [41, 42]. Both Sema3A and Otx-2 bind to the PNNs via the highly sulfated CS-E. Upon binding, Otx-2 is internalized into the cells and regulates the gene expression for the maturation of PNN-positive parvalbumin neurons in the cortex [42, 43]. Binding of Sema3A to PNN-glycans potentiates the inhibitory properties of PNN-glycans to the outgrowth of DRG neurons in vitro [41].
2.2. Class 3 semaphorins

Semaphorins (Sema) are a family of axon guidance molecules during CNS development [44]. The family is divided into eight classes and only five out of these eight Sema are expressed in vertebrates. Unlike other types of vertebrate Sema which are either transmembrane or membrane-anchored, class 3 Sema (Sema3) is the only secreted Sema in vertebrates [45]. To date, Sema3-A, −B, −C, −E, and -F have been identified in an injured CNS [46]. They are produced by the meningeal fibroblasts migrating into the lesion area and up-regulated the expression of different members of Sema3 [46, 47]. The binding of Sema3 to its receptors, neuropilins or plexins, induces a strong growth cone collapse in DRG neurons [48]. Apart from acting through the corresponding receptors, Sema3 also mediate their function though binding to the PNNs. We have previously shown that Sema3A binds to PNNs and that this binding is mediated by a specific CS structure in the PNNs, CS-E. Blocking the binding of Sema3A to PNN glycans reduces the inhibition imposed by PNN to DRG neurons [41].

2.3. Tenascin-C and -R

Tenascin (Tn) family has four members and two of them are expressed in the CNS, including Tn-C and Tn-R [49, 50]. They are both up-regulated after CNS injury [51, 52]. Tn-C is expressed by astrocytes, radial glia, and subsets of developing retinal and hippocampal neurons during early CNS development. In adults, Tn-C is restricted to areas of high plasticity including the olfactory bulb, the cerebellum and the retina. Tn-C interacts with other ECM molecules such as integrins, proteoglycans, and collagen [50]. Tn-C up-regulates after CNS injury and interacts with the different CSPGs in the glial scar. This interaction has been implicated to the failure of axon growth beyond the injury site [53, 54]. Expression of an appropriate integrin isoform, which binds and uses Tn-C as substrate, elicits an enhancement in regeneration [55, 56].

Tn-R is trimer which is expressed in both the developing and adult brains, primarily by neurons, including the horizontal cells from the retina [57]. In adults, Tn-R is found in the PNNs [58, 59]. The trimeric TnR interacts with the core protein in the CSPGs, consolidating the PNN structure [60]. Tn-R has negative influence on axonal growth [49]. Knockout mice of Tn-R, which forms disorganized PNNs, demonstrates enhanced motor recovery after spinal cord injury suggesting that 1) Tn-R is important for PNN structure and that PNNs dissolution enhances plasticity for functional recovery [61].

2.4. Laminins

Laminins are large heterotrimeric glycoproteins that contain an alpha chain, a beta chain and a gamma chain joined together in a coiled–coiled structure. Sixteen isoforms have been identified in vivo, and are differentially expressed both temporally and spatially in various tissues [62]. Genetic disruptions of laminin chains lead disruptions in various tissues and also functional properties in the CNS [63, 64]. The major receptors for laminins are classified as integrins and non-integrins [65]. We shall discuss the role of integrins in later sections of this chapter. For non-integrins receptors such as dystroglycan and GM1 gangliosides, the binding of laminin serves critical functions in the peripheral nervous system (PNS) including myeli-
nation by Schwann cells, neurite outgrowth, and the integrity of blood–brain barrier [66–70]. In the CNS, laminin is primarily present in the basement membrane and is up-regulated by astrocytes after injury [71], although reports of individual isoforms of laminin have also been reported [72]. This suggests an neuronal heterogeneity of laminin isoforms in the adult brain.

Laminin provides a positive guidance to axons during development [73], and act as a supporting substrate to adult retinal ganglion cell [74] and retinal pigment epithelial cells in vitro [75]. The expression of laminin decreases during maturation of the optic system [76] even though, in our recent study, we observed that laminin is still present in adult retinas and optic nerves [74].

2.5. Collagens

Collagens are the most abundant proteins in the animal kingdom, there are now 29 known collagens [77, 78]. A triple-helical organization of component pro- α-chains defines the collagens and 49 distinct collagen α-chain gene products have been described [79]. Collagens are classified into both fibrillar and non-fibrillar forms and can also be assembled into reticular networks and sheets [80]. The organization, distribution, and density of fibrils and networks vary with tissue type and the direction and magnitude of forces to which are given tissue is subjected.

Collagens expressed in the PNS provide a scaffold for the growth and attachment of Schwann cells which also guide the neurite extension [78, 81]. After injury, there is an over up-regulation of collagen which changes the mechanical properties of the lesion area, hinders, and delays regeneration to occur [78]. Collagen is implicated in the progression of glaucoma, a visual neurological disease. One of the characteristics of certain glaucoma is the increment of the intraocular pressure in the anterior chamber of the eye. The heightened pressure is transmitted to the posterior eye chamber, pressing the retinal ganglion cells and eventually driving them to death [82]. Since these cells are the neurons responsible for transmitting the visual signal from the eye to the brain, their cell death leads to inevitable blindness. One of the reasons for the increasing pressure in the anterior chamber is due to an obstruction of the filtering tissue present in the trabecular meshwork, where the aqueous humor flows. The cells of this tissue have the ability to secrete the extracellular matrix. In an attempt to adapt to a biomechanical insult, the cells in the trabecular meshwork increase the synthesis of ECM, including collagen, thus blocking the flow of the aqueous humor and leading to an elevation of intraocular pressure. This mechanism has been proposed as possible cause of the origin of glaucoma [82, 83].

Other important implication of collagens in glaucoma is found in the lamina cribosa, a structure located in the optic nerve head where axons exit from the retina to the optic nerve [84]. A dysregulation in collagen secretion at this point implicates an interruption of axonal transport from the retinal ganglion cell in the retina to the visual areas in the brain. Studies have shown that activated astrocytes are cells responsible for the collagen synthesis and alterations here [85].
The third implication of collagen in glaucoma is the stiffness of the sclera and its implication in the lack of elasticity of the eye [86]. The sclera is the structure that supports the attachment of ocular components including the retina and optic nerve head. A complex network of collagen fibers forms the sclera’s major component and is a major influence on the tissue’s biomechanical response to changes in the intraocular pressure. It has been proposed that the mechanical influence of the sclera may be a key on the eye injury after elevation of the intraocular pressure due to the alterations in the thickness, mechanics and matrix ultrastructure provided by the arrangement of scleral collagen and fiber orientation [87].

### 2.6. Integrins

Integrins are a family of cell surface receptors that are important for cell adhesion to ECM proteins. They are the principal receptors on animal cells for mediating most ECM attachment and signaling. They connect the extracellular environment to intracellular cytoskeleton and are responsible for the activation of many intracellular signaling pathway [88]. All integrins are heterodimeric molecules containing two subunits, α and β. Each αβ combination has its own specificity and signaling properties [89]. Most integrins recognize several ECM proteins. Conversely, individual matrix proteins, such as fibronectin, laminins, collagens, and vitronectin bind to several integrins [90, 91].

The binding of ECM to integrins is regulated by integrin conformation which is determined by the activity inside the cell (inside-out signaling), while upon binding to the ECM molecule, integrin also changes its conformation and elicits signals that are transmitted into the cell (outside-in signaling) [92]. The best understood binding site for integrins is the Arg-Gly-Asp (RGD), which is also found in fibronectin, vitronectin, tenascin, and other ECM proteins. There are 24 types of integrins in humans, formed by the 18 different α-chains and 8 different β-chains, dimerized in different combinations [88]. Each integrin dimer has distinctive properties and functions. Moreover, because the same integrin molecule in different cell types can have different ligand-binding specificities, it is likely that additional cell-specific factors interact with integrins to modulate their binding activity [93]. One example of the variability in integrin expression is in the adult retinal ganglion cells. Cells growing on different ECM in vitro express several distinct combinations of integrins although they are activated and signaled through the focal adhesion kinase pathway [74].

We and the others have previously shown that integrin activation encourages glial cells or neurons to traverse inhibitory areas. Non-specific activation using manganese or specific beta-1 integrin activating antibody, we were able to promote the migration of Schwann cells over CSPG substrate in vitro and encourage axonal outgrowth of DRG neurons [94, 95]. Similarly, integrin activation by over-expressing an integrin mediator kindlin-1 encourages axonal extension on CSPG substrate in DRG neurons in vitro and the growth of DRG neurons passing the lesion site into the spinal cord in vivo [96].

In visual system, integrins are equally important in mediating the cellular response to ECM. Integrin activation using manganese or beta-1 integrin activating antibody enhances the attachment of retinal pigment epithelial cells (RPE) on pathological Bruch’s membrane, which contains a high level of Tn-C, in aged macular degeneration [55]. In retina, we and the others
have also shown that adult retinal neurons have the capacity to grow on multiple ECM substrates including collagen I, collagen IV, fibronectin and laminin with different affinity. This differential binding influences the degree of branching and elongation of neurites [75]. Moreover, we demonstrated that much of effects act through integrins activation.

3. ECM degradation

Matrix components are degraded by extracellular proteolytic enzymes (proteases) acting in the close proximity around the cells after secretion. Many of these proteases belong to two general classes—matrix metalloproteinases (e.g., MMPs and ADAMTSs) and serine proteases (e.g., trypsin, chymotrypsin, elastase) [97–99]. Matrix metalloproteinases represent the largest group with about 50 members identified in vertebrates. Their activity is depended on the binding of Ca$^{2+}$ or Zn$^{2+}$ ions [100, 101]. The second group of matrix degrading enzyme is the serine proteases, which have a highly reactive serine in their active site. Protease activity is generally confined to the cell surface by specific anchoring proteins, by membrane-associated activators, and by the production of specific protease inhibitors in regions where protease activity is not needed [102]. Their activity is important for the homeostasis and turnover of the ECM.

4. ECM implications in retinal pathologies

One of the most specialized forms of ECM is the basement membrane, a flexible, tough, and thin sheet of very well-organized components of the ECM. The functions of basement membranes are to act as platforms for cell adhesion, to provide structural support to a tissue, to divide tissues into compartments, and to regulate cell behavior including polarity. Although small in volume and very thin (typically 40–120 nm), it has a critical role in the architecture of the body [103, 104]. Although the precise composition of the mature basal lamina varies from tissue to tissue and even from region to region in the same lamina, it typically contains the glycoproteins, laminin, type IV collagen and nidogen (also called entactin), along with perlacan [105]. Other common basal lamina components are fibronectin and type XVIII collagen. Interactions of cells with basement membranes are mediated by trans-membrane cell surface receptors, which connect the cytoskeleton of the cell with the extracellular environment, leading to the formation of focal adhesions [88, 106].

The mature polarized retina is structurally and functionally supported by two basement membranes that act as boundaries for the neural retina (Figure 2). The two basement membranes are (i) the Bruch’s membrane, at the interface of the RPE and the choroid and (ii) the inner limiting membrane (ILM) at the interface of the neural retina formed by the endfeet of Müller cells and the vitreous body [107]. Changes in the organization or composition of these basement membranes lead to various pathologies including diabetic retinopathy, age-related macular degeneration, proliferative vitreoretinopathy or retinal detachment [108–111].
Figure 2. Schematic drawing of the cellular components of the retina, glia neurons. The different cell types are placed in the location of a standard large mammalian retina. Note the interactions between the cells and the blood vessels (BV). Amacrine cells (A), astrocytes in green (AS), bipolar cells (B), cones (C), ganglion cells (G), horizontal cells (H), Müller cells in blue (M), microglia in red (Mi), rods (R). Note the location of the different layers of the retina, from the most internal: optic nerve (ON), nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), outer segment layer (OS), pigment epithelium (PE), choroid (Ch). In green the two limiting membranes the Bruch’s and the inner limiting membrane (ILM). In parallel to the drawing electron microscopy pictures of pig retinas showing both membranes.

The Bruch’s membrane is not only providing physical support for RPE, it also regulates RPE differentiation and acts as a barrier that prevents choroidal neovascularization, a process in which choroidal vascular cells inappropriately invade the retina [112, 113]. Alterations in the composition or organization of the Bruch’s membrane compromises the normal function of RPE cells and this disruption results in retinal pathologies including age-related macular degeneration [113].

The ILM lies on the vitreal side of the retina which is the opposite side of the retina from Bruch’s membrane. The ILM constitutes the interface between the retina and the vitreous but is also responsible for organizing and maintaining the laminated structure of the retina and guiding astrocyte migration during vascular development [114]. Disruptions or changes in the ILM are associated with retinal dysplasia as well as retinal pathologies such as diabetic retinopathy, proliferative vitreo-retinopathy [114, 115].

5. Conclusion

This review aims to provide an overview of the major ECM partners and their functions in the CNS, PNS, and retina. As demonstrated above, ECM is not a passive by-stander present in the inter-cellular space, it actively takes part in controlling the penetration and diffusion of molecules in the extracellular space, sequestration and release of chemokines and growth...
factors to neurons, area where it should be permissive and inhibitory to specific population of neurons at a specific time. Dysregulation of their synthesis and production in pathological conditions such as spinal cord injury and glaucoma impede the regeneration in both systems. A better understanding of their spatial and temporal expression, molecular assembly and interaction, and production and degradation will be crucial to harness them for encouraging functional recovery in different pathological conditions.

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