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ABSTRACT

The extracellular matrix is a critical component of every human tissue. ECM not only functions as a structural component but also regulates a variety of cellular processes such as cell migration, differentiation, proliferation, and cell death. In addition, current studies suggest that ECM is critical for the pathophysiology of various human diseases. ECM is composed of diverse components including several proteins and polysaccharide chains such as chondroitin sulfate, heparan sulfate, and hyaluronic acid. Each component of ECM exerts its own functions in cellular and pathophysiological processes. One of the interesting recent findings is that ECM is involved in inflammatory responses in various human tissues. In this review, we summarized the known functions of ECM in neuroinflammation after acute injury and chronic inflammatory diseases of the central nerve systems.

INTRODUCTION

Neurons and glial cells in the central nervous system are tightly associated to each other, maintaining physiological homeostasis of the human body. In addition to the cell-cell interaction, the CNS is also composed of elaborated and complicated extracellular matrixes (ECMs). Neural ECMs are radically different from those of other tissues. The interstitial ECMs are mainly composed of a loose meshwork of hyaluronan, sulfated proteoglycans, and tenascins (1, 2). Perineuronal net (PNN) is a unique ECM structure surrounding the neuronal cell bodies and neurites to stabilize synaptic connections. PNN is also composed of hyaluronans and lecticans which are the meshwork of interconnected hyaluronans and lecticans (aggrecan, brevican, neurocan, and versican) (3, 4) (Figure 2). Neurons and glial cells are both responsible for the production and formation of neuronal ECMs (5). The expression of neuronal ECMs are actively regulated during CNS development. The functions of neuronal ECMs in development and synaptogenesis are extensively studied (6), but the correlations between neuronal ECMs and neuroinflammation are currently under intense research because they are not precisely

understood. Upon the initial damage of CNS tissues by either traumatic injury or degenerative processes, the inflammatory responses in the CNS actively remodel the neuronal ECMs to prevent expansion of neuronal damage or to promote recovery of damaged tissue. These active changes in ECMs not only regulate transcription of genes involved in ECM production, but also modify existing ECM molecules by post translational modifications such as proteolytic cleavages, fragmentation, or release of GAG (glycosaminoglycan) residues from the core proteins. These modifications may either improve the recovery of neuronal damage or may aggravate the inflammatory cycles, leading to chronic inflammation in the CNS.

In this review, we describe the structural composition of neural ECMs and the changes of neural ECMs initiated by damage to the CNS. Also, we summarize the inflammatory reactions regulated by the changes in neural ECMs.

Extracellular matrixes in CNS

Most of the ECMs in other tissues are mainly composed of fibrous proteins such as collagen, fibronectin, and laminin. In contrast, the major components of ECMs in the CNS are proteoglycans, hyaluronan, and tenascins meshwork which are interconnected to each other (1, 2). The ECM in the CNS takes about 20% of the total volume (7) and there are mainly two different types (Figure 1).

Interstitial ECM is loosely distributed in the CNS and mainly composed of proteoglycans and hyaluronic acid with a small amount of fibrous matrix. The other specialized ECM in the brain is perineuronal net (PNN). Most neuronal cell bodies and neurites in the vicinity of the cell body are tightly associated and covered by PNN. PNN is believed to be more than mechanical support for neurons; rather, PNN regulates synaptic plasticity by stabilizing synaptic connections. PNN is also composed of a large amount of hyaluronic and proteoglycans, mainly sulfated proteoglycans and a small amount of fibrous proteins (3, 4). In this review we will summarize the structures and functions of sulfated proteoglycans and hyaluronic acid in neuronal ECM.

Lectican, the sulfated proteoglycan in neuronal ECMs

Lecticans are a class of chondroitin sulfate proteoglycan (CSPG) which share structural features and comprise major ECM networks in the CNS. All lecticans have a conserved binding site to hyaluronan in their N-terminus G1-domain, the central domain which the GAG chain is attached to, and a lectin-containing G3 domain mediating the binding to glycoproteins such as tenascins (8, 9) (Figure 3).

Although the entire lectican family of CSPGs shares structural similarity, lectican may exist as a variety of isoforms based on the chemical composition of GAG chains. The GAG chain is attached to the core proteins in the endoplasmic reticulum (ER) and Golgi compartment (10-13). The glycosylation of lectican proteoglycan is initiated by the addition of xylose residue to a serine in the central domain of lecticans. This reaction is mediated by xylotransferase in ER (10, 11, 13). Next, two additional galactoses are sequentially added to the xylose in the Golgi compartment prior to the addition of GAG chains (14). The GAG chains in CSPGs are repeating disaccharide units composed of N-acetyl-D-galactosamine (GalNAc) and D-glucuronic acid (GlcUA) (15). The length of a GAG chain is variable and more than 100 GalNAc-GlcUA disaccharide can be attached to the central domain of each lectican (15).

Beside the diversity of the length of GAG chains in lecticans, more complexity is due to the sulfation of chondroitin disaccharides. The modifications of chondroitin are mediated by sulfotransferase which adds sulfates on the specific carbons of either GalNAc or GlcUA (16). Based on the position of the sulfation, the CS chain is named CS-A (sulfation on C4 of GalNAc), CS-C (sulfation C6 of GalNAc), CS-D (sulfation on C6 of GalNAc and C2 of GlcUA), or CS-E (sulfation on C4 and C6 of GalNAc). CS-A and CS-C are known to be the most prevalent forms of chondroitin sulfate in the GAG chains of neuronal lecticans.

The functions of CSPG may be very different based on their number, sulfation, and the core proteins in CNS development and function. The diverse roles of CSPGs in normal brain development and functions are reviewed elsewhere.

Aggrecan

Aggrecan is well-known as a major proteoglycan in cartilages (17). It functions to absorb pressure from mechanical loading. In the brain, aggrecan exerts different functions as it is

predominantly observed in the perineuronal net (PNN) around the neuronal bodies and dendrites (18, 19). Neurons and astrocytes express aggrecan (20), which is thought to function on the maturation and stabilization of synaptic connections in the developing brain (19, 21).

As one of the major lecticans in the brain, aggrecan is connected to the hyaluronan via its N-terminus G1 domain, and its C-terminus binds to tenascin as a structural unit of neuronal ECMs (22). There are several suggested functions of aggrecan. Aggrecan secures high-rate synaptic transmission in PN-associated neurons (23), and protect neurons from the oxidative stress through scavenging redox-active cations. (24)

Versican

Versican is the second most dominant neuronal ECM. It is expressed by neurons, astrocytes, and oligodendrocytes (25-27). As with other lecticans, the structure of versican includes an N-terminus G1 domain, C-terminus G3 domain, and central GAG attachment sites. One of the unique properties of versican is that it is expressed as four splicing variants by alternative splicing (28). The central GAG attachment domain is a major splicing site; versican-V0 has the largest GAG attachment sites while versican-V1 and V2 have shorter central domains. The central GAG glycosylation region is spliced out in Versican-V3. The isoforms of versican are expressed in various tissues and Versican-V2 is predominantly expressed in the brain (29). Versican is involved in various cellular functions such as cell migration and inflammation. Especially, during an inflammatory response, versican regulate leukocytes migration and infiltration by integrating with CD44 and Toll-like receptors (30).

Brevican

Brevican is one of the CNS specific lecticans and is expressed in broad regions of the brain (31, 32). Both neurons and astrocytes express it (26). In addition to the secreted ECM components, brevican is also expressed in an alternative spliced form which gives rise to the GPI linked form of proteoglycan. As a structural component of PNN, brevican seems to regulate neuronal plasticity. Brevican was suggested to be involved in CNS injury and Alzheimer's disease (33).

Neurocan

Neurocan is also a CNS specific lectican and is predominantly found in PNN (34, 35). Although the exact function of neurocan in brain function and development is still under research, a genetic association study has suggested that the neurocan gene is associated with human bipolar disorder and schizophrenia (36, 37).

Lecticans and neuroinflammation

The functions of CSPGs in neuroinflammation are most well studied in acute injury and inflammatory responses of the CNS.

The immediate responses to an acute injury of the CNS is the activation of astrocytes (38). Activated astrocytes migrate to the injury site, secrete inflammatory factors, and remodel the neuronal ECMs to prevent the expansion of neuronal damage (39, 40). One of the major outcomes of the inflammatory reaction mediated by activated astrocytes is to construct glial scars comprised of CSPGs. The glial scar functions as a barrier and isolates the damaged region from other tissues (41). This barrier protects neuronal tissues from further blood-driven inflammatory factors such as fibrinogen, TNF- α and IL-1 β (42). The common response of activated astrocytes and infiltrated leucocytes upon neuronal damage is increased expression of several CSPGs such as neurocan, versican, and brevican around the injury (43). In contrast, aggrecan expression was reported to be downregulated. The major signaling molecules modulating CSPG expression is thought to be TGF- β molecules. Once TGF- β molecules leak out from the blood stream, they activate SMAD2 phosphorylation. SMAD2 is known as a critical signal for proliferation and activation of astrocytes. In addition, activated SMAD2 induces CSPG expression from the astrocytes (43). Recent study showed that CSPG expression is differentially regulated by SMAD2 or SMAD3 such that neurocan expression requires both Smad2 and Smad3, whereas phosphacan and chondroitin synthase 1 expression is regulated by Smad2 but not Smad3 (44). Other research, however, reported that TGF- β induces expression of three different CSPGs by PI3K-Akt-mTOR signaling pathway, not by the canonical SMAD signaling pathways (45).

Although the CSPG-mediated changes in ECMs protect the expansion of inflammatory signals from the injury site, increased expression of CSPGs inhibits the axonal regeneration by inducing growth cone collapse (46). This inhibitory function of CSPGs suppresses the recovery

from CNS damages. Furthermore, CSPGs inhibit NSPCs neural progenitor cell migration while, facilitating differentiation of neural progenitor cells into astrocytes (47).

The length and sulfation pattern of GAG seem to have differential effects on the neuroinflammation. For example, CS-A and CS-B GAGs are more potent in promoting the release of inflammatory factors such as IL-1 β , IL-2, and IL-12 (48-50). In contrast to the proinflammatory function, aggrecan derived 6-sulfated CS possesses protective functions from the neuroinflammation by suppressing of NF- κ B nuclear translocation which inhibits the infiltration of T-cells and activation of microglial cells (51, 52). Also, Chondroitin 6-O-sulfate was reported to ameliorates CNS damages in experimental autoimmune encephalomyelitis model (53). However, other research showed that liberated CS may increase proinflammatory cytokines.

CSPGs also modulate chronic neuroinflammatory diseases such as Alzheimer's disease (AD) and multiple sclerosis (MS) (54). CS-GAG was shown to promote the aggregation of A β 1–42 fibrils in vitro (55). Among CSPGs, chondroitin 4-sulfate, chondroitin 6-sulfate, and unsulfated chondroitin were immunostained in senile plaques and neurofibrillary tangles in AD (56). One of the brain specific CSPGs, brevican, is shown to be found in smaller CS side chains (57). In addition, brevican binds to A β 1–42 fibrils (58).

MS is an inflammatory auto-immune disease characterized by gradual loss of myelination in the CNS. Several lecticans such as neurocan, aggrecan, and versican are known to be upregulated around MS lesions (59, 60). CSPGs in the multiple sclerosis are known to facilitate the activity and migration of leucocytes the central nervous system (61). In addition, the polymorphism of the ChGN1 gene, encoding a critical glycosyl transferase for CS production, is shown to be associated with MS progression (62).

Despite conflicting observations on the functions of CSPGs, targeting CSPGs is a promising strategy to enhance neuronal regeneration around the lesion. One of the strategies to control CSPGs in a CNS lesion is to use chondroitinase-ABC (Ch-ABC), which is a bacterial enzyme that liberates CS-GAG from the CSPG core proteins. Treating Ch-ABC in a CNS lesion efficiently reduces the accumulation of CSPGs in the glial scar (63, 64). Furthermore, Ch-ABC treatment promoted axonal regeneration in the CNS lesion and resulted in better recovery in mouse models of spinal cord injury (64, 65). Recent studies showed that intrathecal injection of ChABC increases IL-10 and reduces proinflammatory IL-12 (66). Ch-ABC was also

effectively used to ameliorate AD symptoms in animal models (58).

Another strategy to modulate CSPGs after CNS injury is to inhibit CSPG synthesis by treating β -D-xylopyranosides, an inhibitor for xylose attachment to the serine residue of lecticans.

Treating β -D-xylopyranosides two days after spinal cord injury significantly improved recovery (67). Interestingly, immediate treatment of xyloside after injury inhibited the recovery, indicating that the timing for targeting CSPG is critical for better recovery (67). In addition to the use of xyloside to treat acute lesions, xyloside treated lysolecithin-demyelinated mice increased the number of oligodendrocytes in lesions and improved remyelination (68).

In addition to changing the expression of CSPGs, neuronal injury also triggers fragmentation of CSPGs. Liberated fragments of CSPGs may directly influence the inflammatory responses (69). Fragmented CSPG is known to function as damage associated-molecular pattern (DAMP) and directly binds to pattern recognition receptors (PRR) such as Toll-like receptors (TLR) (70). Indeed, for an example, versican can directly bind to the TLR-2 and activate macrophages (71). These fragmented-ECMs and their pathogenic roles as DAMP molecules are well recognized in other ECM-related disorders such as arthritis (72). The pathogenic function of fragmented CSPGs certainly needs to be studied further in neuroinflammation.

Hyaluronan (Hyaluronic acid; HA)

Hyaluronic acid (HA) is the most abundant GAG in CNS ECMs. Compared to the CSPGs, HA is not sulfated and is not attached to the core proteins (Figure 2). In normal conditions, HA exist as high-molecular weight polymers of repeating disaccharide, D-glucuronic acid, and N-acetyl-D-glucosamine, in neuronal ECM. The average molecular weight of HA is above 1000kDa in normal conditions.

HA polymer is synthesized by HA synthase (HAS1, HAS2, HAS3) (73) and HASs are mainly expressed in the astrocytes in the brain (74). The HA chains serve as scaffolds for other CSPGs to bind and form meshwork of ECMs.

Hyaluronan and neuroinflammation

Beside the structural functions as a major ECM component, HA plays various roles in regulation of neuroinflammation. In normal conditions, high molecular weight HA (HMW-HA) binds to CD44 and reduces TLR mediated inflammatory signals in microglia (75). However, HA functions as a proinflammatory signal in inflammatory situations(76). During the inflammatory responses after injury, HMW-HA is fragmented into low molecular weight-HA (LMW-HA) and this fragmented LMW-HA is released from the damaged ECM (76).

The LMW-HA functions a universal DAMP to induce innate immunity and proinflammatory factors. LMW-HA exerts a proinflammatory role by binding to the CD44 on microglia and astrocytes upon neuronal injury . Furthermore, LMW-HA induces NF- κ B signaling and increases the expression of TNF- α and IL-1 β in cultured neurons (77). Recent studies also suggested that HA concentration in cerebrospinal fluid is correlated to increased blood-brain barrier permeability (78).

Tenascins

There are six family members of tenascins in mammals: tenascin-C, tenascin-R, tenascin-W, tenascin-X, tenascin-Y, and tenascin-N. Tenascin-R is exclusively expressed in the CNS while the others are expressed in a variety of tissues (79).

The structures of tenascins are very similar among the family members. Each tenascin member possesses a cysteine-rich N-terminus domain which mediates the oligomerization of tenascins to functions as a structural component of ECMs (80). Tenascins form oligomers by disulfide bond via this N-terminus oligomerization domain (Figure 3). The central region of tenascin is composed of EGF like repeats and the C-terminus region is composed of fibrinogen III (FNIII) repeats. The C-terminus FNIII repeat domains are known to mediate bindings to other ECMs such as CSPGs.

Beside the structural functions, tenascins influence neuronal differentiation and regulate axonal guidance and neurite outgrowth during brain development (81, 82). Furthermore, tenascins are known to regulate voltage-gated sodium channels and synaptic plasticity (83).

Tenascins and neuroinflammation

Tenascin-C and tenascin-R have been reported to be upregulated after brain injury or spinal cord injury. Reactive astrocytes, along with oligodendrocyte and neurons, are major sources of tenascins expression upon CNS injury (84).

The molecular mechanism by which tenascins regulate immune systems and inflammatory responses are well-studied in non-neural tissues. Tenascin-C expression is increased in response to the inflammatory cytokines such as $\text{INF-}\gamma$, $\text{IL1-}\beta$, and $\text{TNF-}\alpha$ in various tissues (85, 86). The pattern recognition receptor TLR-4 is a major interaction partner of tenascin-C (87) and the expression of TLR-4 is increased after brain injury. The interaction of tenascin-C with TLR-4 promotes the expression of proinflammatory cytokines (88). Consistent with these observations, tenascin-C mutant mice displayed better recovery after experimental brain injury partly by suppressed apoptosis and TLR-mediated proinflammatory responses (89).

In addition to the acute inflammatory responses, tenascin-C was shown to be involve in chronic inflammatory neurodegeneration such as multiple sclerosis (MS) and Alzheimer's disease (AD). MS is an autoimmune disorder in CNS developed by progressive loss of myelination. One of the direct causes of demyelination in MS is the integrin- $\alpha 9\beta 1$ -mediated autoimmune response. The c-terminus FNIII domain of tenascin-C contains integrin- $\alpha 9\beta$ binding motif AEIDGIEL (90), and promotes integrin- $\alpha 9\beta 1$ -mediated cytokine expression in experimental autoimmune encephalomyelitis (EAE) models (91). EAE is a widely used experimental model for MS study. Furthermore, an integrin- $\alpha 9\beta 1$ specific tenascin-C mutant promoted proinflammatory cytokines and this induction was abolished by treating integrin- $\alpha 9\beta 1$ neutralizing antibody (91).

Chronic inflammation may also be responsible for AD and recent studies showed significant correlation in tenascin-C staining to A β plaques in human AD brain samples.

Compared to tenascin-C, tenascin-R function in neuroinflammation is not well understood. However, it was reported that tenascin-R is also upregulated after CNS injury. Further study needs to be performed to elucidate tenascin-R function in neuroinflammation.

Conclusion

The functions of ECM in inflammation have long been studied in human disorders such as fibrosis, cancers, and rheumatoid arthritis. Recent studies provide a substantial amount of

experimental and clinical data indicating that ECM is critically involved in both acute and chronic inflammatory responses in the CNS. However, the complexity of ECM composition and diverse modifications in disease conditions hinder clear understanding of the molecular functions of neuronal ECM in neuroinflammation. Also, the functions of ECM in neuroinflammation change dramatically upon its modification such as fragmentation and alternative splicing. However, several experimental data targeting to modulate ECM to promote CNS injury suggested that neuronal ECM is a promising target to develop therapeutics. Among the most immediate challenges is to elucidate the molecular mechanism of each ECM component and its variants regulating neuroinflammation in CNS. The molecular interactions among ECM and inflammatory signals are under intensive investigation currently. Especially, the clinical data on the efficacy of ECM modifying enzymes and chemicals treating neuroinflammation must be accumulated.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. The extracellular matrices in central nervous systems

Interstitial ECM is loosely distributed in the CNS and neuronal cell bodies and are tightly associated and covered by PNN. Neural ECMs are mainly composed of proteoglycans and hyaluronic acid with a small amount of fibrous matrix

Figure 2. Hyalactan in neuronal ECMs

Neural ECMs are mainly composed of hyalactans which are the meshwork of interconnected hyaluronans and chondroitin sulfate proteoglycans such as aggrecan, brevican, neurocan, and versican.

Figure 3. Lectican family of chondroitin sulfate proteoglycan

Lecticans are a class of chondroitin sulfate proteoglycan (CSPG) including Aggrecan, Versican, Neurocan, and Brevican. All lecticans have a G1-domain which is a conserved binding site to hyaluronan, the central GAG attachment domain, and a lectin-containing G3 domain mediating the binding to glycoproteins such as tenascins.

Figure 4 Structure of Tenascins

- A. The domain structure of major tenascin, Tenascin-C and Tenascin-R.
- B. Tenascins form oligomers and the N-terminus of tenascin meditates oligomerization.

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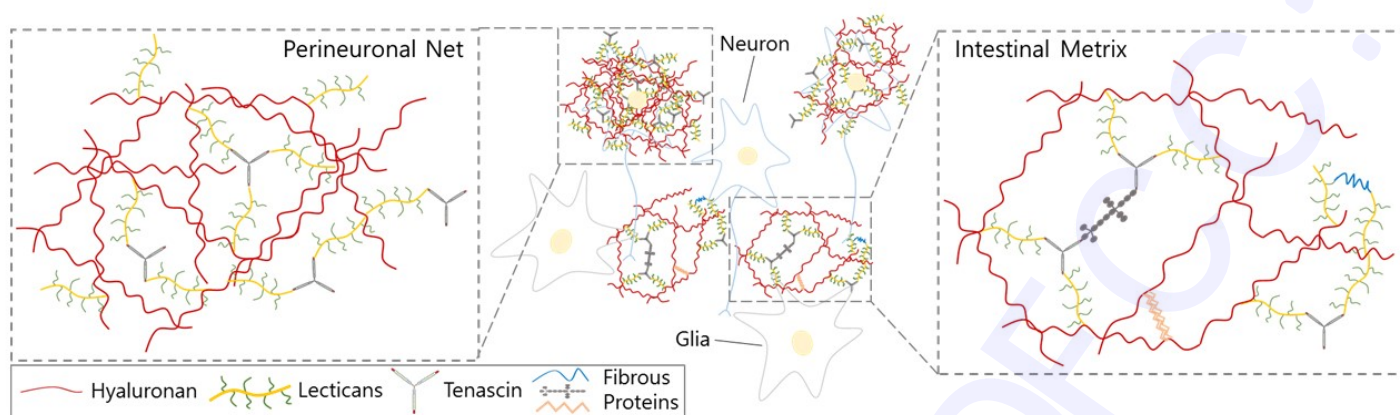
Figure 1

Fig. 1. Figure 1

Figure 2

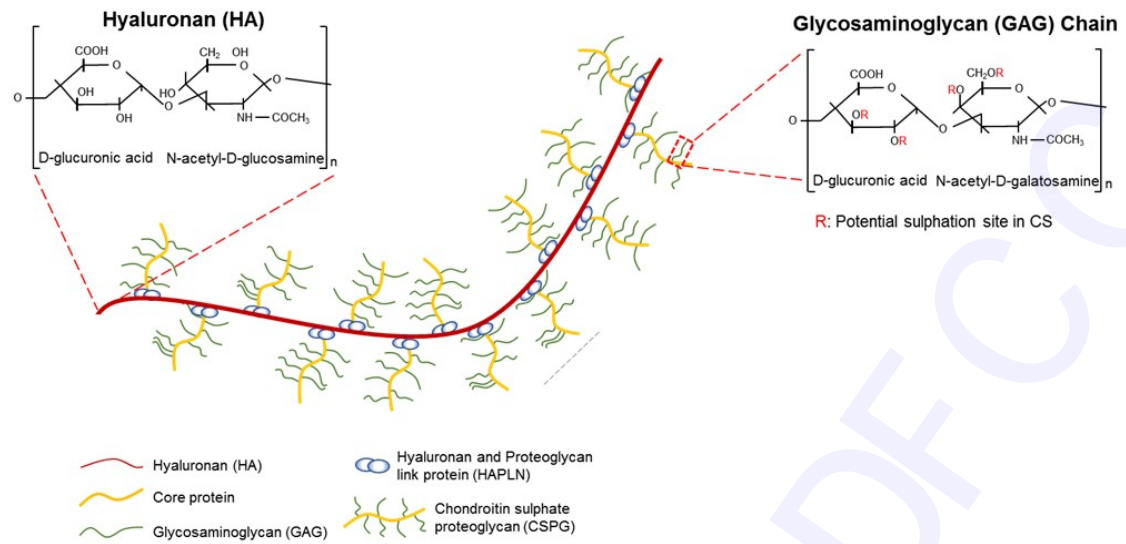


Fig. 2. Figure 2

Figure 3

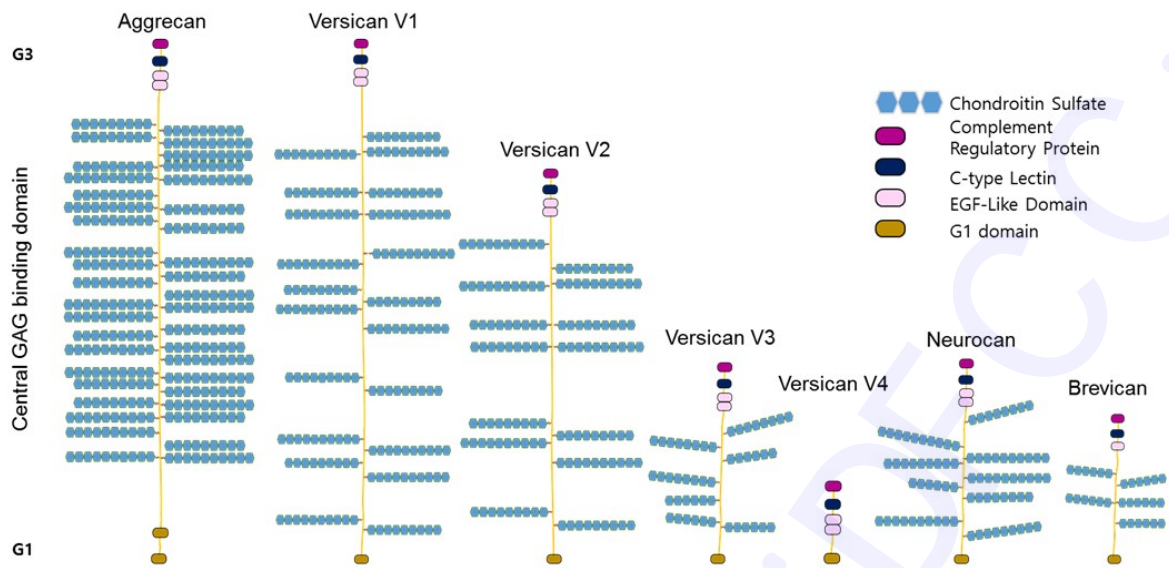


Fig. 3. Figure 3

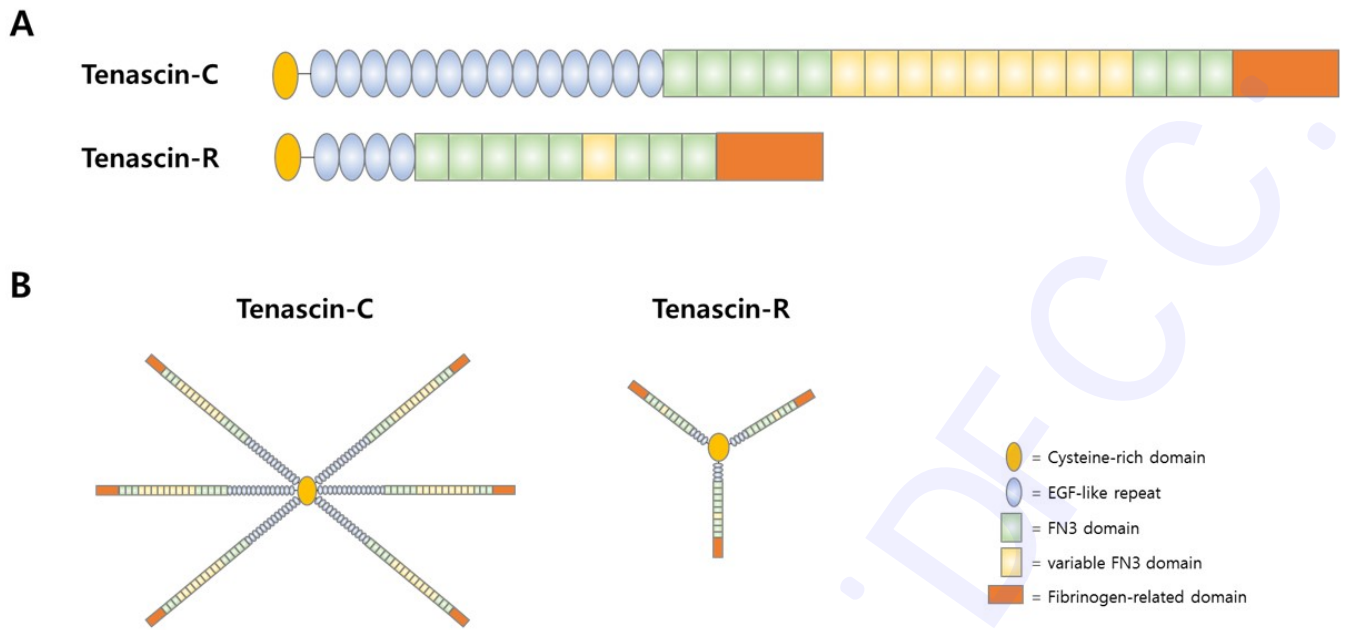
Figure 4

Fig. 4. Figure 4