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ALZHEIMER'S DISEASE AND HEPARAN SULFATE PROTEOGLYCAN

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TABLE OF CONTENTS

1. ABSTRACT

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder. Cardinal histopathologic changes of AD are neurofibrillary tangles (NFTs) and deposits of beta-amyloid protein (A-beta) in the form of neuritic plaques (NPs). Several different mutations found in patients with familial AD have been demonstrated to increase A-beta production, resulting in a common pathological cascade of beta-amyloidosis in the brain. Heparan sulfate proteoglycan (HSPG) has been co-localized with both A-beta in the NPs and NFTs. The proteoglycans are a family of complex macromolecules consisting of a protein core to which glycosaminoglycan (GAG) chains are covalently attached. HSPG has been shown to bind to A-beta, accelerate its fibril formation, and maintain its fibril stability. In AD and other neurodegenerative disorders, tau becomes hyperphosphorylated hence it is unable to bind to microtubules which results in the production of paired helical filaments, a building unit of NFTs. It has been shown *in vitro* that sulfated GAGs induce the formation of paired helical-like filaments under physiological conditions from tau. Furthermore, an interaction between HSPG and apolipoprotein E (a potent risk factor of AD) has been shown to be involved in neurodegeneration. Thus, substantial evidence exists to underscore important roles of HSPG in the etiology of AD.

2. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the progressive loss of memory and cognitive functions. Approximately 4 million Americans suffer from this disease and the cost for its care exceeds 80 billion dollars annually. Cardinal pathological changes found in the brain of patients with AD are neurofibrillary tangles (NFTs), mainly composed of highly phosphorylated tau, and deposits of aggregated beta-amyloid protein in neuritic plaques (NPs) and cerebral vessels (cerebrovascular amyloid angiopathy). Important roles of beta-amyloid protein (A-beta) and beta-amyloid precursor protein (APP) in the pathogenesis of AD are underscored by discoveries of missense mutations in the gene encoding APP in a small subset of families with an autosomal dominant form of AD (1). All of these APP mutations increase A-beta secretion, particularly A-beta 1-42(consisting of 42 amino acid residues) (2-6). A-beta 1-42 has been demonstrated *in vitro* to be highly amyloidogenic compared with smaller forms consisting of 39 or 40 amino acid residues (A-beta 1-39 or 1-40) (7). Two additional genes whose mutations give rise to AD have been identified. The most common form of early onset type of AD has linkage to the presenilin-1 (PS1 or S182) gene on Chromosome 14 (8). The mutations found in the presenilin-2 gene (PS2, STM-2 or E5-1) on Chromosome 1 are thought to lead to the development of another, rather rare, type of early onset AD (9, 10). Secreted A-beta 1-42 increases in the plasma and in the media from cultured skin fibroblasts of patients with the PS mutations. Additionally, the increases in secreted A-beta 1-42 caused by the PS mutations have been demonstrated by overexpression of the mutant PS cDNAs in cultured cells and transgenic mice (11-13). Families with PS1 mutations show abundant A-beta plaques (14, 15). Thus, several distinct mutations found in familial AD seem to cause a common pathological cascade of beta-amyloidosis in the brain.

A severe loss of large neurons in the nucleus basalis of Meynert, a basal forebrain cholinergic system (16), hippocampus, and cortex is also characteristic of AD pathology. Since loss of neurons gives a straightforward explanation for the decline of cognitive functions in AD, investigators have been searching for the specific causes of the neurodegeneration. NFTs are thought to be a marker for neurodegeneration and to play a crucial role in its pathogenesis. On the other hand, the concept that A-beta, specifically the fibrillar form of A-beta, is toxic to neurons,

has become widely accepted. Indeed, there has been a long-lasting debate regarding roles of amyloid deposits and NFTs in the neurodegeneration in AD (17). Thus, the mechanisms by which the pathological lesions (NPs, NFTs, and neuronal loss) develop and which relate the pathological lesions have yet to be established.

Aging and apolipoprotein E (apoE) are widely accepted as the most potent risk factors of AD. Prevalence and incidence of AD increase during aging at the rates doubling approximately every 5.1 years (18, 19). ApoE is a key protein of lipid metabolism that is found on several types of lipoprotein particles including chylomicrons, very low density lipoproteins, and high density lipoproteins. There are three common alleles (E2, E3, and E4) for the apoE gene in human. The association of apoE4 allele and AD was first reported by Strittmatter *et al.* (20) and confirmed by other investigators (21-24). Corder *et al.* (25) reported that risk for AD increased from 20% to 90% and the mean age at onset decreased from 84 to 68 years with increasing number of apoE4 alleles in 42 families with late onset AD (Late-onset AD is usually defined as occurring at more than 60 years of age).

The two hallmarks of AD pathology, amyloid deposits and NFTs, contain both HSPG (26, 27, 28, 29) and apoE (30). In addition, HSPG and apoE are implicated in the lipid metabolism and survival of neurons. Thus, heparan sulfate proteoglycan (HSPG) may relate the cardinal pathological lesions in AD. Here, we review the roles of HSPG in the pathogenesis of AD.

3. DISCUSSION

3.1. Chemistry of heparan sulfate proteoglycan

The proteoglycans (PGs) are complex macromolecules consisting of proteins to which polysaccharide chains (glycosaminoglycans, GAGs) are covalently attached. The polysaccharide chains in the proteoglycan consist of repeating disaccharide units of hexosamine (either D-glucosamine or D-galactosamine) and hexuronic acid (either D-glucronic acid or L-iduronic acid) with the exception of keratan sulfate, where hexuronic acid is replaced with a galactose residue. Based upon specific disaccharide units, six distinct classes of glycosaminoglycans are known: hyaluronic acid, chondroitin sulfate (CS), dermatan sulfate, heparan sulfate (HS), heparin, and keratan sulfate. Heparan sulfate proteoglycans (HSPGs) are found on cell surfaces and in the extracelluar matrix of all mammalian tissues. HS consists of an unbranched chain of repeating disaccharide units containing glucosamine and hexuronic acid. Glucosamine residues are at least two types of amyloid plaques: neuritic plaques and diffuse plaques. Diffuse plaques are Congo red negative but immunohistochemically recognized by antibodies against A-beta. Diffuse plaques are thought to be precursors of neuritic plaques. The concept that diffuse plaques are associated with little local cytopathology is supported by ultrastructural observations (39, 40) and by the use of quantitative synaptophysin immunohistochemistry to demonstrate that diffuse plaques have synaptic densities indistinguishable from the surrounding A-beta-free cortical neuropil, whereas neuritic plaques have significant decrease in synaptic density (41). Diffuse plaques in the cerebral cortex and hippocampus are also immunoreactive for HSGAG (26, 42, 43). Although Snow *et al.* (43) reported positive staining of diffuse plaques with HSPG core protein antibody, Van Gool *et al.* (42) found no immunoreactivity of diffuse plaques for HSPG core protein. This discrepancy may be due to the differences in antibodies and, although less likely, patients, used in their experiments. In contrast to (GlcN) may be N-sulfated (NS) or N-acetylated (NAc) and may be O-sulfated at C-6. Hexuronic acid can be either D-glucronic acid (GlcUA) or L-iduronic acid (IdUA) and IdUA may be O-sulfated at C-2. Therefore, the repeating disaccharide units of HS are [4-GlcUA beta 1,4-GlcNAc (or NS) (± 6S)-alpha 1]$_n$ and [4-IdUA (± 2S)-alpha 1,4-GlcNS (± 6S)-alpha 1]$_n$. Thus HS is structurally similar to heparin. HS, however, contains lower contents of N-sulfates, O-sulfate, and IdUA, and more GlcUA than does heparin. HS is attached to serine residues at Ser-Gly sequences in a protein core through a tetrasaccharide linkage region consisting of 4-GlcUA-beta1,3-Gal-beta1,3-Gal-beta1,4-Xyl-beta-O-Ser, where Gal and Xyl represent D-galactose and D-xylose, respectively. Generally two to four HS chains are attached to a protein core and CS may be found in some protein cores (31). HSPGs found in the brain are dystroglycan (32), N-syndecan (33) glypican (34), and perlecan (35). HSPGs differ in their protein cores that are thought to determine the location of HSPG in the cell membrane (syndecan and glypican) and extracellular matrix (perlecan and dystroglycan). The composition of HS isolated from brain differs significantly from that of other organs and is developmentally regulated (36). Thus, differences in protein cores, disaccharide composition, the extent and position of sulfation, and the number of GAG chains diversify chemical structure and functions of HSPGs. The postulated functions of HSPG include cell proliferation, differentiation, adhesion, migration, and morphogenesis.

3. 2. Chemical composition of heparan sulfate in Alzheimer's disease

Are there any alterations in HSPG structure in AD? Using skin fibroblast cultures derived from AD patients and control subjects, Zebrower *et al.* (37) found that GlcUA beta 1,4-GlcNS was increased by 30% in the medium from AD skin fibroblast cultures and that GlcUA beta 1,4-GlcNS-6 was reduced by 40% in AD (37) and postulated that HS in AD brain might be altered and that this might be caused by abnormal control of O-sulfotransferases in HS biosynthesis (38). Lindahl *et al.* (36), however, reported no significant structural difference between HS obtained from AD and control brain tissues. Although their report indicated a slight elevation in N-sulfation for some of the AD cerebral HS preparations.

3.3. Immunohistochemical localization of heparan sulfate proteogycan

Several groups localized HSPG and HSGAG to NPs, cerebrovascular amyloid, and NFTs by immunohistochemistry (26, 27, 29) and by a basic fibroblast growth factor (bFGF) binding study (28). There

diffuse plaques in the cerebrum, those in the cerebellum are not reactive to antibodies against either HSPG core protein or its GAG (43). Snow *et al.* (43) speculated that the reason for the scarcity of neuritic plaques in the cerebellum compared with the cerebrum was the lack of HSPG in diffuse plaques in cerebellum. Further experiments are needed to determine if HSPG in the cerebrum is different from that in the cerebellum in terms of its amount, structure, and metabolism. Perlecan has been identified as a major HSPG present within A-beta plaques (26, 43).

Does every neuritic plaque contain HSPG or HSGAG? Su *et al.* (29) reported occasional findings of A-beta positive neuritic plaques that did not show HSGAG-immunoreactivity and of HSGAG-positive plaques that did not react with A-beta antibodies. Recently novel plaque-like structures (AMY plaques) in AD brain were reported by Schmidt *et. al* (44). AMY plaques do not contain A-beta. Therefore, it is interesting to consider a possible relationship

between AMY plaques and HSGAG-positive plaques lacking A-beta and a possible role of HSGAG in formation of AMY plaques.

3.4. Beta-amyloid protein and its precursor protein

A-beta, a 4 kDa peptide, was first isolated from cerebral blood vessels from AD and Down syndrome patients (45, 46). The A-beta that accumulated in AD brains is heterogeneous at its C-terminus, resulting in peptides of 39-43 amino acids (figure 1). Diffuse senile plaques were shown to consist predominantly of A-beta 1-42/43 by using C-terminal specific anti-A-beta antibodies, whereas classic senile plaques contained both A-beta 1-40 and A-beta 1-42/43, as well as shorter A-beta peptides that were truncated at the N-terminus (47, 48). Cerebrovascular amyloid contains similar A-beta contents as classic senile plaques (47). A-beta may exist in a soluble form as well as in a beta-pleated sheet conformation. Residues 14-21 and 29-39/42 of A-beta possess the beta-sheet forming properties (49). A-beta 1-42 is less soluble and forms fibrils faster than shorter isoforms *in vitro* (50), indicating the C-terminal sequence of A-beta is critical in determining the solubility of the peptide. It has been hypothesized that the amyloid deposition *in vivo* may be seeded by A-beta 1-42 fibrils and grow rapidly by assembly of A-beta 1-40, which is the most abundant isoform produced by cultured cells (4). Therefore, even a slight increase in the amount of A-beta 1-42 might be sufficient to lead to beta-amyloidosis (51, 52).

A-beta is derived from a larger transmembrane glycoprotein, APP, by proteolytic processing (figure 1). APP is encoded by a gene consisting of 18 exons located on chromosome 21 at 21q21.2(53). Exons 16 and 17 of the APP gene encode in part for A-beta (54). The complete sequence of ~300 kb of the human APP gene has been reported recently by Hattori *et al.* (55). Three major isoforms containing the A-beta sequence are produced by alternative splicing of the APP gene primary transcript and are designated as APP695, APP751, and APP770. The APP695 isoform consisting of 695 amino acid residues is expressed predominantly in neurons, whereas the APP751 and APP770 isoforms are expressed ubiquitously and include exon 7 which encodes a domain similar to a Kunitz protease inhibitor (56). The APP770 isoform composed of 770 amino acid residues additionally contains exon 8 that is similar in sequence to the MRC OX-2 antigen (57). Other isoforms of APP (consisting of 752, 733, 714, 696, and 677 amino acid residues) are also produced in a much smaller amount by alternative splicing. APP belongs to a multi-gene family of APP-like proteins, but APP is the only one which comprises the A-beta region (58).

As illustrated schematically in figure 1, APP is processed through different proteolytic pathways. The proteolytic enzymes involved in these pathways have not yet been identified. The putative alpha-, beta-, and gamma-secretases may each represent a collection of enzymes. Alpha-secretase cleaves the APP molecule at the cell surface between residues 16 and 17 of A-beta, releasing a soluble form of APP (59). This pathway is thought to be non-amyloidogenic since intact A-beta is not produced. Generation of A-beta requires cleavage of APP by beta- and gamma-secretase at the N-terminal and C-terminal ends of A-beta, respectively. It was believed previously that A-beta generation was an abnormal event resulting from a pathological condition. Later it was shown that intact A-beta was released into the medium of normal cultured cells and that soluble A-beta could also be detected in cerebrospinal fluid of both normal individuals and AD patients (60), indicating that A-beta production is part of a physiological process. The processing of APP can be altered, however, under pathological conditions. Mutations in 3 different genes implicated in the etiology of familial AD have been shown to increase A-beta production (APP, PS1, and PS2) (2-6, 11-13) and the presence of the apoE4 allele has been shown to increase A-beta deposition (61, 62). These findings suggest that the distinct gene alterations and the apoE4 allele lead to the common pathological cascade of beta-amyloidosis.

3.5. Beta-amyloidosis and heparan sulfate proteoglycan

3.5.1. Interaction between beta-amyloid protein and heparan sulfate proteoglycan

APP binds with high affinity to HSPG from the basement membrane before and after treatment with heparitinase digestion to remove its GAG side chains (63). A-beta also binds to both the core protein and GAG side chains of vascular HSPG with high-affinity(64). This HSPG is likely to be perlecan since perlecan has a similar binding profile (65) and since perlecan is a major HSPG found in the basement membranes including those in vasculature. APP binding to HSPG, particularly to perlecan, is mediated by a heparin binding consensus sequence (VHHQKL) located only in the A-beta portion of APP (65, 66). A-beta easily self-aggregates to form amyloid fibrils *in vitro*. The binding of heparan and heparin to A-beta (11-28 and 1-28) enhances aggregation (67, 68). The interaction of these sulfated polysaccharides with A-beta is pH-dependent with increasing interaction as the pH values fall below neutrality. One or more histidine residues in a consensus heparin-binding domain of A-beta must be protonated for this interaction to occur and mediate the interaction because the pH profile of heparin-induced aggregation of A beta(1-28) has a midpoint pH of approximately 6.5 (68). Perlecan accelerates A-beta fibril formation more rapidly than other amyloid plaque components, such as apolipoprotein E, alpha 1-antichymotrypsin, amyloid P component, compliment C1q, and C3. Perlecan also stabilizes these amyloid fibrils once formed. These effects of perlecan on amyloid fibril formation and stabilization are primarily due to the property of its GAG side chains (69). Thus, HSPG, particularly perlecan, promote both amyloid fibril formation and stabilization.

Because of the limited availability of HSPG and the structural similarity of heparin to HSPG, heparin has been widely used to study roles of HSPG in AD and other diseases. APP binds to heparin with a high affinity and the presence of micromolar zinc(II) increases in the affinity for heparin two- to four-fold (70). Clarris *et al.* (71) demonstrated that, using deletion mutants of APP695 and synthetic peptides homologous to candidate heparin binding domains, APP contains at least, four heparin-binding domains (residues 96-110, 131-166, 316-346, 382-447 of APP695) and excluded a heparin binding consensus domain of A-beta. Heparin binds to the fibrillar form of A-beta but not the non-fibrillar form (72), indicating that the aggregation state of A-beta rather than its primary sequence governs its heparin-binding properties. This is also supported by demonstration of a similar heparin binding property with the Dutch mutant form (figure 1) of A-beta (glutamine for glutamic acid substitution at the 22nd residue of A-beta found in Dutch families with hereditary cerebral hemorrhage with amyloidosis) and amylin (another amyloidogenic protein and its deposits found in islets of Langerhans in diabetes type II) (72). Thus, the binding nature of heparin to A-beta and APP is quite different from that of HSPG, particularly, perlecan. Precautions should be taken when the roles of HSPG are deduced from the results obtained from use of heparin.

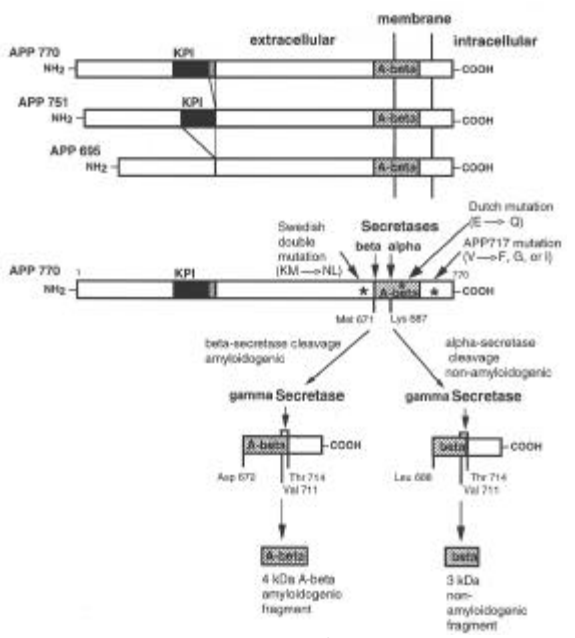

Figure 1. Schematic structure of APP and its processing by proteases. APP is a transmembrane protein and three major isoforms of APP are expressed. The larger two isoforms consisting of 751 and 770 amino acid residues (APP751 and APP770, respectively) contain a Kunitz protease inhibitor (KPI) domain and are ubiquitously expressed. The APP695 isoform does not contain a KPI domain and is predominantly expressed in neurons. All three forms are thought to undergo similar proteolytic degradation to generate a 4 kDa beta-amyloid protein (A-beta) and a 3 kDa non-amyloidogenic protein. The 1st and 43rd amino acid residues of A-beta correspond to Asp 672 and Thr 714 in APP770, respectively. The approximate locations of mutations found in patients with familial Alzheimer's disease are indicated by asterisks.

3.5.2. Rat infusion models of brain amyloidosis

The importance of HSPG in the accumulation and persistence of A-beta deposits in AD brain was highlighted by rat infusion models created by Snow *et al* (73). They infused rats directly into hippocampus with 1) A-beta plus perlecan with GAG, 2) A-beta only, 3) perlecan with GAG only, 4) vehicle only, 5) A-beta plus GAG of perlecan, and 6) GAG of perlecan only. One hundred percent of animals receiving A-beta (1-40) plus perlecan with GAG showed Thioflavin S and Congo Red positive deposits (indicative of amyloid fibril formation) at the infusion site and 50% of the animals infused with A-beta only, demonstrated Congophilic deposits at the infusion site. All such deposits immunohistochemically contained both the core protein of HSPG and GAG while Congo Red and Thioflavin S negative sites in the remaining animals infused with A-beta only were negative for HSPG and HS GAG immunoreactivity. Neither Congo Red nor Thioflavin S staining was observed in any animal infused with either vehicle only, perlecan only, GAG only or A-beta plus GAG.

3.5.3. Processing of amyloid precursor protein and heparan sulfate proteoglycan

Treatment of PC-12 cells (a cell line of rat pheochromocytoma) with p-nitrophenylxyloside, known to inhibit proteoglycan formation, increases the non-amyloidogenic cleavage of APP by alpha-secretase (74). Consistent with this observation, heparin induces an increase in the secretion of total APP, and a much greater relative increase in the secretion of beta-secretase cleaved APP (amyloidogenic cleavage) in a human neuroblastoma cell line (75). These data support the hypothesis that highly sulfated heparan sulfate proteoglycans may promote amyloidogenic pathways of APP metabolism. One explanation for these observations is that both heparin and HSPG may bind to a presumptive heparin binding consensus domain (VHHQKL), comprising amino acid residues 12-17 of A-beta, and subsequently protect APP from alpha-secretase cleavage. The proposed consensus amino acid sequence for heparin binding is XBBXBX where Bs represent basic amino acid residues (76). However, this argument contradicts the fact that heparin does not bind to monomeric or non-fibrillar A-beta *in vitro* at physiological pH (68, 71, 72). HSPG and heparin seem to promote amyloidogenic cleavage of APP but the molecular mechanisms leading to an increase in A-beta production remain to be elucidated.

3.5.4. Beta-amyloid clearance and heparan sulfate proteoglycan

HSPG and chondroitin sulfate proteoglycan (CSPG) inhibit the proteolytic degradation of fibrillar, but not non-fibrillar, A-beta at physiological pH *in vitro* by binding to fibrillar A-beta. This binding is thought to be due to sulfated carbohydrate moieties of the GAG chains (77). Rat cortical neurons and meningeal fibroblasts cannot degrade A-beta in culture media. In contrast, microglia and astrocytes digest A-beta and microglia remove A-beta fibrils immobilized on culture dishes (78). Astrocytes exposed to A-beta fibrils secrete proteoglycans which inhibit removal of A-beta fibrils by microglia (78). It remains to be identified which proteoglycans from astrocytes have the inhibitor activity.

3.6. Neurite outgrowth and heparan sulfate proteoglycan

Nerve growth factor increases the APP secretion from cultured hippocampal cells. Secreted APP stimulates neurite outgrowth of cultured neurons (79). The binding of secreted APP to HSPG in the extra-cellular matrix is thought to be essential to this neurotrophic effect and one of the heparin-binding domains of APP (residues 96-110) has been identified as a potential binding site for this function (79, 80). Thus, HSPG may play a role in both neurodegeneration and neuroregeneration.

3.7. Neurofibrillary tangles and heparan sulfate proteoglycan

Also Alzheimer described the first case of AD as a tangle disease (the English translations of his original report appear in references 81 and 82). Indeed, the topology and abundance of NFTs in the brains of AD patients relate better to the dementia than do the plaques (83, 84). NFTs are also present in neurons in other neurodegenerative diseases and NFTs are considered to be a marker of neurodegeneration. NFTs mainly consist of paired helical filaments (PHF) assembled from tau protein. Tau is one of the microtubule-associated proteins and is found in the axonal segment of peripheral and central neurons. Tau can assemble microtubules into bundles when introduced into nonneuronal cells (85, 86). The tau gene is localized to chromosome 17 in human (87), and at least 6 isoforms are generated by alternative splicing of the single gene transcript. In AD, the six tau isoforms become abnormally phosphorylated and form the paired helical filament (88). Phosphorylation of tau is developmentally regulated, and the status of tau phosphorylation influences stability of microtubules (89). Tau protein from PHF can not bind to

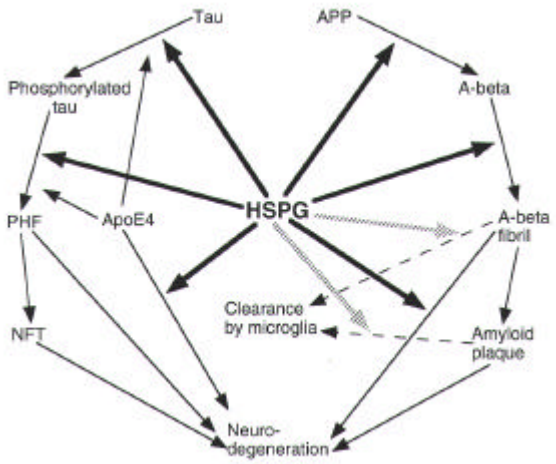

Figure 2. Possible roles of heparan sulfate proteoglycan in the pathogenesis of Alzheimer's disease. HSPG may increase the production of beta-amyloid protein (A-beta), accelerate formation of A-beta fibrils, and maintain stability of the A-beta fibrils, leading to neurodegeneration (indicated by bold arrows). HSPG may inhibit clearance of A-beta fibrils by microglia (striated arrows). HSPG may stimulate tau phosphorylation and induce the formation of paired helical filaments (PHF) (bold arrows) HSPG may interact with apoE and lead to neurodegeneration.

microtubules (90). Therefore, it has been postulated that hyperphosphorylation of tau leads to formation of PHF and NFTs, resulting in degeneration of neurons. The mechanism for hyperphosphorylation of tau in AD remains to be clarified, however.

HS is also co-localized with extracellular and intracellular NFTs in neurodegenerative diseases including AD by immunohistochemistry and the bFGF binding assay (26, 28; 91-93). Sulfated GAGs induce formation of paired helical-like filaments under physiological conditions *in vitro* from non-phosphorylated tau (94) and this effect seems to be proportional to the degree of GAG sulfation. That is, highly sulfated GAGs such as dextran sulfate, pentosan polysulfate, and heparin are the most potent inducers while non-sulfated GAGs such as dextran and hyaluronic acid have no effect and moderately sulfated GAGs such as HS, chondroitin sulfate, and dermatan sulfate show intermediate effects (95). Heparin stimulates tau phosphorylation, inhibits binding of tau to taxol-stabilized microtubules, and promotes disassembly of microtubules made from tau and tubulin (94). It is possible that HS has similar effects as heparin on the phosphorylation of tau and the disassembly of microtubules.

Recently, a novel autosomal dominant disease called familial "multiple system tauopathy with presenile dementia" has been described (96). Abundant fibrillary deposits of tau protein in both neurons and glial cells but no amyloid deposits characterize the pathology. The tau deposits (twisted filaments) are different from that of PHF in diameter and periodicity. However, HS is immunohistochemically co-localized to the deposits. HS may play a role in formation of tau fibrils in this disease as well.

3.8. Apolipoprotein and heparan sulfate proteoglycan

Interaction between apolipoprotein and HSPG is the first step necessary for the uptake of apolipoprotein E-containing lipoprotein mediated by LDL (low density lipoprotein) receptor-related protein (LRP) on the cell surface of hepatocytes *in vitro*. This HSPG/LRP pathway also mediates lipoprotein uptake in neurons (reviewed in 97). In the presence of lipoproteins, apoE4 but not apoE3 inhibits neurite outgrowth of cultured dorsal root ganglion neurons and neuroblastoma cells. This differential effect of apoE3 and apoE4 on neurite outgrowth of cultured neuronal cells is abolished by chlorate, lactoferrin, and receptor-associated protein. These molecules block the HSPG/LRP pathway (98). Crutcher and colleagues (99, 100) also demonstrated that synthetic peptide containing amino acid residues 141-155 of apoE and the 22 kDa N-terminal thrombin-cleavage fragment of apoE causes degeneration of neurites from embryonic chick sympathetic ganglia *in vitro* with the fragment derived from apoE4 being much more toxic than that from apoE3. They also showed that this toxicity is prevented by addition of heparin, heparan sulfate, sodium chlorate and heparinase, the LRP receptor-associated protein, and a polyclonal anti-LRP antibody, all of which may alter the HSPG/LRP pathway. These results suggest that the HSPG/LRP pathway is involved in apoE associated neurodegeneration and the remodeling of damaged neurons in AD and that HSPG may play a role in the apoE4-associated pathogenesis of AD.

4. PERSPECTIVE

As reviewed above, HSPG plays significant and multifactorial roles in the pathogenesis of AD (summarized in figure 2). HSPG has been co-localized to NPs and NFTs, the two hallmark histopathologic lesions of AD. HSPG has been shown to bind A-beta and accelerate its fibril formation and maintain its stability, thus augmenting the formation of amyloid deposits in AD. Furthermore, HSPG appears to promote the amyloidogenic cleavage of APP resulting in a greater abundance of A-beta. In addition, *in vitro* studies have shown HSPG to react with tau protein to promote its hyperphosphorylation and aggregation, the net result being the formation of PHFs and thus NFTs. Also, it has been shown that HSPG interacts with apoE and, in the presence of apoE4, specifically, this interaction results in neurodegeneration.

Important studies remain to be performed to further clarify the roles of HSPG in AD pathology. Although HSPG has been immunohistochemically localized to NPs and NFTs, its biochemical isolation from these structures has yet to be performed. This process would allow for the identification of the proteoglycan core protein(s), the number of GAG chains, and the extent and position of GAG sulfation. The determination of the chemical structure of HSPG is a necessary step toward a complete understanding of the roles of HSPG in the etiology of AD and should make new experimental approaches possible. This may also facilitate (i) dissecting possible alterations in HSPG metabolism in AD, (ii) relating NFTs and amyloid deposits, and (iii) resolving discrepancies in immunohistochemical localization of HSPG core protein in diffuse plaques. A further understanding of the roles of HSPG in the etiology would assist in the development of rational, targeted therapeutic strategies to combat this significant disease.

Transgenic animal technology should prove to be a valuable tool to further understand the roles of HSPG in the pathogenesis of AD. What kind of transgenic mice would be feasible to test the HSPG hypothesis in AD? Currently, it is not known if the metabolism of HSPG is altered (upregulated, altered molecular structure, synthesis, degradation, etc.) in individuals who develop AD. Although

it has been shown that no difference was apparent in the perlecan mRNA levels between individuals with AD and unaffected controls (101), perlecan specifically seems to be a prime HSPG candidate playing a role in AD pathology. Transgenic mice overexpressing a murine perlecan core protein construct have been created and are currently under investigation (102). The genes encoding the enzymes modulating GAG sulfation and attachment to the core protein need to be cloned and characterized in order to produce transgenic and knockout mice. Effects of over- and underexpression of such genes in transgenic mice on beta-amyloidosis and neurofibrillary changes can be tested by mating with transgenic mice overexpressing APP (103-106) and tau (107). Such transgenic mice will be useful as screening tools for therapeutic and preventive agents of AD.

5. ACKNOWLEDGMENTS

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Key words: heparan sulfate, proteogycan, Alzheimer's disease, amyloid, amyloidosis, beta-amyloid protein, perlecan, amyloid precursor protein, neurofibrillary tangles, neuritic plaques, tau, apolipoprotein, protein processing, etiology, and immunohistochemistry

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