Blood–Brain Barrier Degradation and the Implication of SPARC Protein as a Potential Therapeutic Target for Alzheimer's Disease

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Abstract: Alzheimer's disease is a progressive neurodegenerative disorder affecting a substantial portion of the older population, with the number of afflicted individuals expected to grow with time. Although numerous contributing factors to the disorder have been identified, there is currently no cure or effective prevention method. With the situation as dire as it is, many efforts have been made to shed light on the mechanisms tying diverse contributing factors to the pathogenesis of Alzheimer's disease. One common neuropathological feature of Alzheimer's disease is the dysfunction of the blood–brain barrier, which normally maintains brain homeostasis by isolating it from the peripheral circulation and mediating the transport of various bloodborne elements in and out of the brain. An increase in the blood–brain barrier permeability has been observed in Alzheimer's disease at a level considerably above normal aging. This chapter provides an overview of the effects of aging, the neuroimmune system, inflammation, traumatic brain injury, apolipoprotein E gene ε4 allele, and secreted protein acidic and rich in cysteine (SPARC) protein on blood–brain barrier. The potential

of SPARC as a therapeutic target for Alzheimer's disease, and the application of deep-learning-based virtual screening tools against SPARC protein are explored.

Keywords: Alzheimer's disease; blood–brain barrier; Hevin; secreted protein acidic and rich in cysteine; virtual screening

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that has widespread detrimental effects on memory and cognitive abilities that worsen over time. The disease is ultimately fatal, often through complications associated with decreased cognition. It is the most common form of senile dementia, with an estimated 5.8 million Americans currently afflicted with AD, a number that is expected to increase dramatically with an aging population that is more consistently reaching the "oldest-old" phase where AD risk is at its highest (1). AD has a substantial economic impact, with projections indicating the global cost of dementia could balloon to 2 trillion US dollars by 2030 (2). Given the threat the disease poses, researchers have been tackling AD from different angles, but as of now attempts to develop treatments have been met with widespread clinical failure (3).

The blood–brain barrier (BBB) consists of endothelial cells, serving as a layer of separation between blood vessels and the brain. The endothelia that line the blood vessels of the brain serve to isolate the brain parenchyma from bloodborne molecules that lack corresponding transporters to mediate their entry, and maintain the equilibrium of the brain's environment. The barrier is also comprised of other elements interacting with endothelial cells, including astrocyte foot processes and pericytes, which together with neurons and microglia comprise the neurovascular unit. The BBB is also responsible for controlling immune surveillance within the brain, by restricting the flow of immune cells (4). Dysfunction of the BBB is implicated in AD pathogenesis. The BBB is partially responsible for the clearance of amyloid-beta (Aβ), which builds up and forms plaques in AD, and the BBB is a site of CNS inflammation, which is frequently observed in AD patients (5).

While the integrity of the BBB is tightly regulated, emerging evidence implicates the matricellular proteins, secreted protein acidic and rich in cysteine (SPARC), and Hevin, as having a role in regulating BBB permeability. This chapter discusses the effects of various AD risk factors on BBB permeability, with emphasis on SPARC, which is upregulated in AD brain tissue (6, 7). Since the SPARC protein enhances BBB permeability, promotes neuroinflammation, and prolongs pro-inflammatory M1 phase of microglia, its potential as a druggable target is also discussed.

THE BBB IN AD

The integrity of the BBB is critical to the maintenance of brain homeostasis in health. As mentioned, BBB dysfunction is commonly seen in cases of AD, and a variety of factors may contribute to the observed disruption.

Aging

Age is perhaps the most predominant risk factor for AD, with almost half of all individuals over the age of 85 suffering from it; conversely, less than 10% of cases under the age of 65 suffer from AD (8). BBB deterioration has been well-documented in aging (9), with nearly all its components being affected (Table 1). Degradation of the BBB is known to start early, with notable permeability increases around the hippocampal region in individuals between 23 and 47 years of age, which worsens with increasing age (9, 10). Hormones such as insulin, which are associated with aging, can affect the permeability of the BBB and contribute to leakage (11). Additionally, transporters of certain molecules such as glucose, along with various proteins and hormones, may become defective in older individuals, reducing their availability to the brain (12). Outside of the barrier, the appearance of white matter hyperintensities, which are indicative of a loss of vascular integrity, also correlates with age. Damaged vasculature may cause a corresponding decrease in BBB integrity (13). Corroborating this, the vascular density of the brain appears to experience a significant age-related decline between the ages of 57 and 90 (14). Hypertension, which is more common in older individuals, can contribute to microvascular injury, thereby increasing the incidence of BBB disruption (15, 16).

TABLE 1	Alterations of BBB components during physiological aging
BBB element	**Property changes due to aging**
Endothelial cells	Increased capillary wall thickness
	Decreased number of endothelial cells
	Decreased number of mitochondria
Tight junctions	Decreased expression of tight junction protein
Basal lamina	Increased thickness of basement membrane
	Increased concentration of collagen IV and arginase
	Decreased concentration of laminin
Astrocytes	Increased astrocyte proliferation
	Increased GFAP expression
Microglia	Changes to amoeboid morphology
	Production of neurotoxic proinflammatory mediators
Pericytes	Degeneration and loss of pericytes
	Vesicular and lipofuscin-like inclusions
	Increased size of mitochondria
	Foamy transformation
Neurons	Deterioration of synaptic plasticity
	Deficit in long-term potentiation
	Impaired neurogenesis
	Increased apoptosis
	Neuronal damage due to cytokine release

Adapted from (9). GFAP, glial fibrillary acidic protein; BBB, blood–brain barrier.

Neuroimmunity

The brain exists in a state that is considered "immune privileged." Due to the existence of barriers between the brain and the rest of the body's circulation, the brain is insulated against many peripheral immune events (17). The presence of immune cells derived from main circulation, such as peripheral macrophages, neutrophils, and leukocytes, in the brain is an indication of BBB breakdown (18). In addition, the brain has intrinsic immune components, and these components interact with the BBB in such a way that peripheral immune events can also invoke a response in the brain (17). There are two primary types of neuroimmune cells, microglia and astrocytes, and both interact with the BBB.

Microglia are a variant of macrophage, though they do not develop and function as peripheral macrophages do. Microglial progenitors emerge from the yolk sac, and the development of microglia occurs in phases, with each phase being regulated by different transcription factors, and exhibiting differing gene expression profiles (19). Some of their key functions include phagocytosis, synapse pruning, and mediating immune signaling through the release of cytokines and other factors (20). They play a role in AD primarily by phagocytizing abnormal Aβ amyloid and forming a barrier between the plaques and the rest of the brain through plaque envelopment, thereby limiting the expansion of the plaque (21). Microglia are known to associate tightly with the BBB. Microglia exist in a resting state until they are activated due to brain injury or another immunological stimulus. Upon activation, they release a host of cytokines and other molecules that increase the permeability of the BBB; in the case of brain injury, this allows bloodborne agents like myeloid cells to cross the BBB. They also have been found to release reactive oxygen species that impair the function of the BBB (22). Perhaps, the most significant contribution of microglia to AD pathology is their involvement in evoking inflammatory responses within the brain (23). The role of inflammation in BBB disruption is discussed further in the following section.

Astrocytes are of epithelial origin and feature a wide array of morphologies and functions within the nervous system. Aside from their roles in neural immunity, they are responsible for ion transport, removal and catabolism of neurotransmitters, and neurogenesis. Some astrocytes are noted for their vascular end-feet, which are closely associated with brain vasculature and the BBB (24). They increase the permeability of the BBB through vascular endothelial growth factor A (VEGFA) and thymidine phosphorylase (TYMP). VEGFA, along with the TYMP product 2-deoxy-d-ribose, downregulates tight-junction proteins and promotes angiogenesis and BBB permeability (25). The release of these two factors is induced by interleukin-1 beta (IL-1β), an inflammatory cytokine (25). Both astrocytes and microglia produce and react to inflammatory responses that can impact BBB health primarily through inflammatory cytokines (26).

Neuroinflammation

Neuroinflammation has been found to be relevant to AD pathology in a variety of ways. Inflammation in the brain has widespread effects on vasculature, cell signaling, neural function, and other immune responses. The effect of cytokines and other inflammatory mediators released during an inflammatory event involve some of the key components of the neuroimmune system and have been found to regulate the

clearance of Aβ (27). Many of these mediators have also been shown to influence BBB permeability. A summary of some of the mediators and their effects on the BBB are given in Table 2 (28). Neuroinflammation is a common effect of aging and notably includes an increase in the production of inflammatory cytokines by microglial cells (29). These mediators, in general, are not directly responsible for modulating the permeability of the BBB. Instead, they influence the expression or activation of other factors that, in turn, disrupt the BBB function (30).

Traumatic brain injury

Traumatic brain injury (TBI) has been shown to be a significant risk factor for AD. Individuals who had experienced a mild traumatic brain injury (mTBI) are more vulnerable to early-onset cognitive impairment (31) than those that have not experienced such an event. The effects of a TBI are often immediate, with force-induced injury resulting in what is considered secondary brain damage, which includes an increase in BBB permeability. Following TBI and mTBI, bloodborne substances accumulate in various regions of the brain, due to a breach in the BBB. While, in most cases, the effects of mTBI appear to be relatively short-lived, in rats with preexisting hypertension, mTBI can induce persistent disruption of the BBB (32). These rats experienced an increase in fibrin accumulation and neuronal expression of inflammatory cytokines (32). Generally, a focal breach following mTBI has been observed in rats to persist for

TABLE 2	Inflammatory mediators and their effect upon the BBB
Inflammatory mediator	**Observed effects on BBB**
TNF-α	Increase in BBB permeability in *in vivo* and *in vitro* models
	Increased efflux of albumin from brain to blood
	Decreased ZO-1 expression
	Increased MMP-9 protein expression
IL-1β	Increase in BBB permeability in *in vivo* and *in vitro* models
	Decreased TEER of primary cultures of brain endothelial cells and human brain endothelial cells
	Increased production of PGE and COX
	Decreased ZO-1 expression
IL-6	Decreased TEER in cerebrovascular endothelial cells from rats at higher doses but not at lower doses
	Decreased BBB permeability in ischemic brain in rodents
IL-17A	Increase in BBB permeability in *in vivo* and *in vitro* models
CRP	Increase in BBB permeability in *in vivo* and *in vitro* models
	Increase in ROS production in brain endothelial cells

Adapted from (28). BBB, blood brain barrier; COX, cyclooxygenase; CRP, c-reactive protein; IL, interleukin; MMP-9, matrix metalloproteinase-9; PGE, prostaglandin E; ROS, reactive oxygen species; TEER, transepithelial/transendothelial electrical resistance; TNF-α, tumor necrosis factor alpha; ZO-1, zonula occludens-1

approximately 24–48 h (33, 34). In rare occurrences, singular instances of BBB disruption via mTBI in humans, typically measured by the cerebrospinal fluid/ serum albumin quotient, have been found to persist for months or even years (35). A meta-analysis of studies conducted from 1995 to 2012 found that TBI and mTBI events are substantial risk factors for AD (36). The BBB disruption at the onset of TBI is relatively short-lived; however, the subsequent events lead to structural degeneration in the brain causing long-lasting cognitive impairments (37). Disruption of the BBB has been observed to be a marker of mild cognitive impairment independently of the neurofibrillary tangles (NFTs), tau protein, and Aβ amyloid plaques, indicating that substantial breakdown of the BBB itself contributes to cognitive decline in addition to exacerbating other neurodegenerative processes in AD (38).

Apolipoprotein E gene ε4 allele (APOE ε4)

The APOE ε4 allele has been identified as the most significant genetic risk factor for AD (39). It is also associated with other dementia subtypes, such as Parkinson's (40) and frontotemporal dementias (41, 42). Individuals homozygous for APOE4 (ε4/ε4) experience a 10-fold higher risk of dementia, and individuals heterozygous (ε3/ε4) for the variant experience a 1.7-fold higher risk of dementia (43). APOE4 is attributed to reduced clearance of Aβ amyloid, which contributes to the formation of the Aβ amyloid plaques that are a hallmark of AD (44). Possession of at least one APOE4 allele increases the leakage of the BBB (45, 46). The role of APOE in maintaining the integrity of the BBB is confirmed by experiments involving APOE deficient (APOE$^{-/-}$) mice, which exhibit signs of increased BBB permeability, such as the leakage of exogenous tracers, starting at 2 weeks old (18). The allele may also have relevance to TBI. When assessing the BBB repair ability of APOE3 and APOE4 mice, the APOE3 mice experienced a significant reduction in permeability between the 3-day and 10-day measurements, indicating substantial BBB repair. APOE4 mice, however, did not experience a significant reduction in permeability in the same time period; APOE4 was also expressed at lower levels than APOE3 at both 3 and 10 days (47). The role of APOE in BBB integrity is further reinforced by postmortem studies of both AD and normal humans with and without the APOE4 allele. AD-afflicted APOE4 carriers experienced a 3.1-fold increase in fibrin perivascular deposits in the brain relative to APOE3 carriers, indicating an increase in BBB permeability. The same study also found that APOE3 carriers still had a 6.9-fold increase in Aβ amyloid deposits relative to normal controls, indicating that BBB disruption is indeed a significant component of AD. In addition, pericytes, which are constituents of the BBB, have substantially reduced coverage in both AD and normal individuals (48).

SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE

SPARC belongs to a family of matricellular proteins that modulate cell interaction with the extracellular environment. There are currently six known members of the SPARC family. These members, along with some key features of them, are shown

in Table 3. While their structures and functions are not identical, each member of the family possesses shared motifs and is secreted into the extracellular space where they influence the structure of the extracellular matrix and modulate various signaling pathways (58) such as the TGF-β pathway (30, 58). Two particular members of this family, SPARC and Hevin/SPARCL1, are notable in that they have collagen binding domains in addition to the calcium binding domains exhibited by all of the other members of the protein family (30). The SPARC-collagen binding interaction is depicted in Figure 1. Nullification of SPARC expression decreases the expression of the proinflammatory cytokines IL-6, IP-10, and FAS/CD95 in rats (59). Hevin is a member of the SPARC protein family, which is most commonly expressed in the brain along with SPARC (30). Studies have found that SPARC has an antiadhesive effect on brain endothelial cells and decreases cerebral endothelial transepithelial/transendothelial electrical resistance (TEER), indicating decreased BBB integrity (60).

SPARC expression in AD

Within the brain, SPARC and Hevin are attributed to a variety of functions, such as the regulation of synaptogenesis and tissue remodeling following an injury. The proteins are primarily expressed in immune cells. While both SPARC and Hevin are produced by microglia (7) and astrocytes (61), Hevin is produced only by some neurons (62). Postmortem examination of the brains of AD and control individuals found that there is a notable upregulation of SPARC and downregulation of Hevin in the AD brains. As indicated in Figure 2, SPARC is expressed by microglia found in close proximity to pathological Aβ amyloid plaques (7). Interestingly, while it seems that SPARC has a destructive effect on the BBB, which would exacerbate the AD condition, it appears to support the Aβ amyloid clearance process, which should have the opposite effect (7, 60). Hevin's role in BBB health is unclear, though it may be responsible for the initiation of the repair process by microglia (7).

TABLE 3	Members of the SPARC protein family		
SPARC family member	**Significant brain expression**	**Binds to**	**Impact on cell adhesion**
SPARC	Yes (7, 30)	Collagen + calcium (30)	Antiadhesion (49, 50)
Hevin/SPARC-like 1 (SPARCL1)	Yes (7, 30)	Collagen + calcium (30)	Antiadhesion (51)
Smoc-1	Yes (52)	Calcium (30)	Unidentified
Smoc-2	No (53)	Calcium (30)	No effect on non-epithelial cells (53)
Testicans/spocks	Yes (54, 55)	Calcium (30)	Antiadhesion (testican-1) (56)
Follastatin-like 1 (FSTL1)	Yes (57)	Calcium (30)	Unidentified

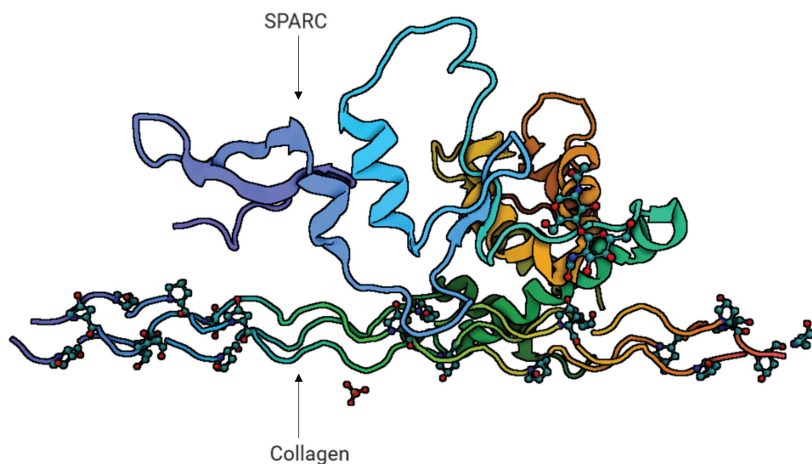SARC, secreted protein acidic and rich in cysteine.

Figure 1. **Secreted protein acidic and rich in cysteine (SPARC)-collagen binding.** The bottom part of the structure diagram represents a collagen alpha-1(III) chain; the top part of the structure diagram represents SPARC. The SPARC-collagen binding site represents a potential target for agents that modify SPARC activity. *Created with BioRender.com*.
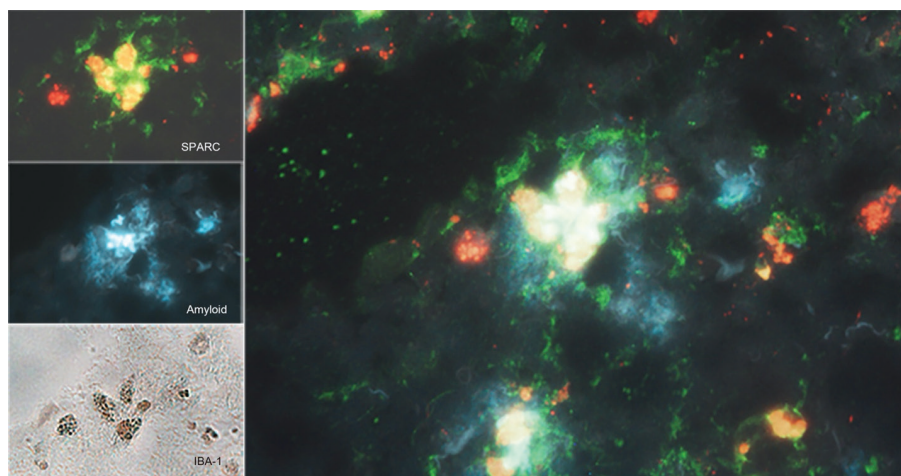

Figure 2. **Secreted protein acidic and rich in cysteine (SPARC) is expressed by microglia found in close proximity to pathological Alzheimer's Aβ amyloid aggregates.** Analysis of cortical tissue from Alzheimer's disease patients reveals the presence of SPARC (in green) in and around glial cells within Aβ amyloid (ThioS in blue) plaques. These SPARC-associated glial cells were identified as microglia (IBA-1 in HRP brown). Adapted from (7) under CC BY-NC 4.0 (https://creativecommons.org/licenses/by-nc/4.0/) license. Reproduced with permission.

SPARC and the BBB

Expression of SPARC is also associated with inflammatory responses. When testing the effects of various cytokines on SPARC expression and BBB permeability in hCMEC/D3 cell culture, it was found that TNF-α caused an upregulation in SPARC only in the absence of IFN-γ that negated the effects of TNF-α (63).

In the brain, SPARC is typically localized to astrocytic end-feet and cerebral endothelium. SPARC was experimentally determined to increase transendothelial permeability and affect the differentiation of endothelial cells through protein tyrosine kinase signaling (63). A particular area of interest is the SPARC-collagen binding domain. Increased levels of collagen IV, as well as general thickening, in the basement membrane of brain microvessels are noted in cases of AD (64); increases in SPARC show a corresponding increase in the levels of collagen (65, 66), and SPARC acts as a chaperone for collagen IV (67). Furthermore, abnormalities in the vasculature that surround the Aβ amyloid plaques are associated with aberrant levels of collagen IV (68). The interactions between SPARC and collagen have been linked to inflammation and pathological fibrosis (69), as well as induction of a pro-inflammatory response in brain monocytes (64). The differential effects of SPARC may mean it can serve as an effective broad-spectrum therapeutic target.

Rationale for SPARC protein as a potential Alzheimer's therapeutic target

Although a better understanding of the disease and its mechanisms have provided avenues for druggable targets, attempts to develop effective ways to treat or reverse AD progression have been met with failure thus far. Perhaps targeting AD from multiple treatment angles may be the key. As vascular dysfunction is a substantial component of AD, SPARC and other members of the protein family may be druggable targets for AD (70). Information on SPARC modifiers on the central nervous system is limited; however, such modifiers have been studied to a degree in the context of cancers (71, 72). Currently, the translational aspects of these drugs for AD are largely speculative. The SPARC-collagen binding site represents a reasonable start to the search, given the detailed research surrounding the structure and mechanism of the SPARC-collagen binding domain and knowledge of collagen binding with other molecules (73). A general diagram of how SPARC and Hevin, in particular, interact with the BBB is shown in Figure 3.

FUTURE DIRECTIONS

Research on the SPARC protein and molecules that can modify its activity is limited. Given that the process of drug discovery is capital-intensive and time-consuming, it may be prudent to establish what molecules modify the activity of SPARC and its relative such as Hevin. High-throughput screening has been applied to other molecules, in which batteries of mini-scale experiments assaying the activity of the target when introduced to a library of molecules are conducted (74). However, conducting this procedure on a compound library that can contain hundreds of thousands of molecules, with a generally low hit rate of modifying compounds, is costly; samples of every compound must first be synthesized before it can be tested. The use of screening tools that conduct the filtering of molecules *in silico* has become increasingly popular, as large numbers of molecules can be processed quickly and cheaply, so long as one has access to sufficient computational power (75). Molecules must be first tested both *in vitro* and *in vivo* and, ultimately, in humans before a drug
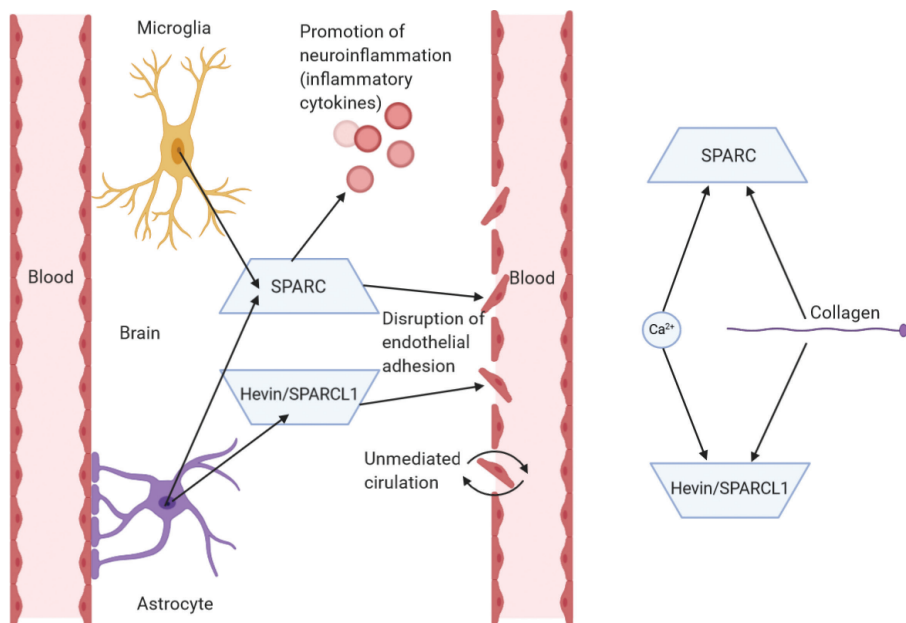
Figure 3. Secreted protein acidic and rich in cysteine (SPARC) and Hevin interactions with the blood–brain barrier (BBB). SPARC is produced primarily by microglia and astrocytes, while Hevin is primarily produced by astrocytes. SPARC and Hevin exert antiadhesive effects, and SPARC promotes the release of inflammatory cytokines in certain conditions. Both SPARC and Hevin are distinct from the other members of the SPARC protein family because they bind to collagen as well as calcium; both binding sites represent potential targets for Alzheimer's disease-modifying agents. *Created with BioRender.com.*

can be considered successful. The fast *in silico* screening compound hits can significantly reduce the time and costs of drug development (76).

We thus propose that such methods be used to identify inhibitors for SPARC-collagen binding. A variety of machine learning (ML) algorithms have been applied to the problem of drug discovery and molecular screening with considerable success; decision trees, support vector machines, and other classifiers have been applied to either structural or ligand-based virtual screening (VS) (77–79). Ligand-based approaches take the similarities of different molecules to other compounds that are known to be active against a target protein. Information on the molecules is generally taken from compound databases, which are filtered based on certain properties, such as those that influence pharmacokinetics and toxicity, in order to make the problem of screening a large number of molecules more computationally feasible (76). As the name implies, structure-based VS involves structural information, either obtained from techniques such as X-ray crystallography or, more commonly, data obtained from computational models. The molecular structures of the protein target and those of the structural databases are examined, in order to determine which will interact in the desired manner (76). Structural methods also encompass the development of novel molecules,

as generative models, which are notable for their ability to use information gleaned from training data for the purpose of classification or prediction and create novel data for a novel sample of the given type.

Deep learning (DL), which involves ML algorithms that feature multiple neural network (NN) layers, has become prevalent in a variety of fields. NNs form the primary basic structure of DL models. Notable examples of NN-based drug discovery platforms include "AtomNet," developed by Wallach et al., a structure-based virtual screener based on a convolutional neural network (CNN) algorithm. Though typically applied to image processing and linguistic applications, a CNN model, which features layers of feature-reduction (convolution) and pooling operations, was trained on a set of molecular structures and tested against a set of benchmark decoy-structures. Performance of the CNN was found to be better than other ML methods (80). Thus, it may be the case that more research into some unorthodox NN strategies may provide a helpful performance boost for VS tools; such a tool could be useful in VS against SPARC protein target for AD drug discovery, as the limited data on SPARC target make *in silico* methods a practical predecessor to future *in vitro* and *in vivo* work and beyond.

CONCLUSION

Current evidence shows that the BBB plays a crucial role in a variety of neurological disorders, and its disruption is evident in AD. There is a great deal of interplay between the various known hallmarks of AD, such as the buildup of amyloid plaques, NFTs, and BBB degradation. Many risk factors tie into multiple facets of the disorder; APOE4, the most significant genetic risk factor for AD discovered to date, diminishes Aβ clearance and inhibits BBB repair. TBI/mTBI and neuroinflammation contribute to AD pathogenesis and BBB damage. Gradual erosion of the BBB is a common part of the aging process, increasing an individual's vulnerabilities to further breakdown and neurodegenerative diseases. Given the importance of the health and stability of the BBB, and the wide array of factors that can be detrimental to it, such as SPARC, more research into its mechanics, maintenance, and recovery pathways may be vital to understanding AD and how to treat it. DL-based VS tools may be employed to identify inhibitors of SPARC-collagen binding for AD drug discovery.

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