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REVIEW

Microtubule affinity-regulating kinases are potential druggable targets for Alzheimer's disease

Narendran Annadurai[1] · Khushboo Agrawal[1] · Petr Džubák[1] · Marián Hajdúch[1] · Viswanath Das[1]

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Abstract Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects normal functions of the brain. Currently, AD is one of the leading causes of death in developed countries and the only one of the top ten diseases without a means to prevent, cure, or significantly slow down its progression. Therefore, newer therapeutic concepts are urgently needed to improve survival and the quality of life of AD patients. Microtubule affinity-regulating kinases (MARKs) regulate tau-microtubule binding and play a crucial role in neurons. However, their role in hyperphosphorylation of tau makes them potential druggable target for AD therapy. Despite the relevance of MARKs in AD pathogenesis, only a few small molecules are known to have anti-MARK activity and not much has been done to progress these compounds into therapeutic candidates. But given the diverse role of MARKs, the specificity of novel inhibitors is imperative for their successful translation from bench to bedside. In this regard, a recent co-crystal structure of MARK4 in association with a pyrazolopyrimidine-based inhibitor offers a potential scaffold for the development of more specific MARK inhibitors. In this manuscript, we review the biological role of MARKs in health and disease, and draw attention to the largely unexplored area of MARK inhibitors for AD.

✉ Viswanath Das
viswanath.das@upol.cz

[1] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 5, 77900 Olomouc, Czech Republic

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Introduction

The last few decades have seen a tremendous life-span improvement due to better healthcare; however, escalating population and unhealthy lifestyles have brought in new challenges to age-related disorders in the elderly population. Alzheimer's disease (AD) is one of the most prevalent forms of age-related disorders, clinically characterized by dementia and a progressive loss of mental, behavioral, and functional decline of the brain. It has become the leading cause of death in elderly over the age of 60 compared to other diseases like cancers, heart diseases, stroke, and others [1].

Historically, the primary hallmarks of AD are the formation of Hirano's bodies, neurofibrillary tangles (NFTs), senile plaques, and cortical Lewy bodies [2–6]. Extracellular senile plaques of amyloid-beta (Aβ) mainly accumulate in the neocortical areas followed by deposition in allocortical regions, entorhinal cortex, and limbic structures [4, 5]. In the advanced stages, Aβ deposits are also observed in the subpial surface of the cerebral cortex [4]. However, unlike Aβ, the intracytoplasmic NFT deposits of hyperphosphorylated microtubule-associated protein (MAP) tau show a conventional pattern of accumulation [4]. NFTs first start to accumulate in the perirhinal and entorhinal regions of the cortex followed by CA1 region of the hippocampus, limbic system, and isocortical areas (reviewed extensively in Ref. [4]).

Tau is primarily found in abundance in axons of neurons and plays a key role in regulating microtubule (MT) dynamics, axonal growth, and a number of other MT-

dependent neuronal functions [7, 8]. Under normal conditions, the physiological functions of tau are tightly regulated by alternating cycles of phosphorylation and dephosphorylation [9]. However, a dysfunction in this cycle is believed to be responsible, in part, for the pathological alterations in AD [10, 11]. While tau hyperphosphorylation is the most widely studied post-translational modification, tau can also undergo a number of other modifications that alter its MT-binding affinity [12]. Protein kinases that play a major role in tau hyperphosphorylation include glycogen synthase kinase 3 (GSK-3), cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), and MT affinity-regulating kinases (MARKs) [11, 13, 14]. Among these tau kinases, MARKs have recently gained significant interest due to their role in Aβ- and tau-mediated toxicity. Although MARKs have also been associated with cancers and metabolic disorder, to limit the scope of this review, we mainly focus on their association to tau pathology in AD. In addition, we present an update on small molecule inhibitors of MARKs that have the potential to develop into promising lead candidates.

Biological functions of MARKs

Microtubule affinity-regulating kinases belong to the calcium/calmodulin-dependent protein kinase superfamily and phosphorylate tau at AD-specific Ser262 site on KXGS motif located in the MT-binding domain (Fig. 1a) [15]. The human MARK family consists of four members (MARK1–4; Fig. 1b) that play a major role in a number of physiological processes [16–22]. MARK4 is an unusual member of the MARK family and lacks a hydrophobic pocket adjacent to the ATP-binding domain [23]. Alternate splicing of *MARK4* gene results in the expression of two additional isoforms (MARK4L and MARK4S) that differ in carboxyl termini [19]. MARK1 and MARK2 expression levels are higher in fetal than adult tissues presumably due to the active proliferation of cells in developing tissues that require highly dynamics MTs [24]. MARK4L is highly expressed in testis, brain, kidney, liver, and lungs [25]. Although the expression of MARK4L remains relatively unchanged in postmitotic cells, upregulation of MARK4L in hepatocarcinomas and gliomas implicates MARK4 in neoplastic transformation [19, 26]. In contrast to MARK4L, MARK4S is predominantly expressed in the central nervous system (CNS) [25], in addition to other non-CNS tissues, such as testis and heart [27].

MARKs regulate key cellular functions

Overexpression of MARK2 and MARK4 in rat hippocampal neurons results in tau hyperphosphorylation, and loss of dendritic spines and synaptic markers [28, 29]. Similarly, MARK1 and MARK2 when overexpressed in Chinese hamster ovary CHO phosphorylate endogenous MAPs, causing their detachment from MTs [30]. Heterologous overexpression of MARK4S in hepatocytes reduces their viability [26]. In contrast, blocking MARK2 activity inhibits neurite outgrowth and differentiation in mouse neuroblastoma N2a cells, indicating the role of MARK in regulating neuronal plasticity [31]. This is also evident following knockdown of MARK2 that induces axonal growth in rat hippocampal neurons [32]. Co-transfection of MARK with Par-3/Par-6/atypical protein kinase C (aPKC) reverses the toxic effect of MARK on axonal growth, presumably due to the inactivation of MARK effect on tau phosphorylation [32]. These data indicate that optimal level of MARK protein and activity is crucial for normal cellular functions.

Trinczek et al. show that in contrast to MARK kinase (MARKK) that shows cytoplasmic distribution, MARK4 co-localizes with MTs [15]. Proteomic analysis of protein complexes from cells suggests a potential interaction between MARK4 and γ-tubulin [33]. Furthermore, co-localization of MARK4 with clathrin-coated vesicles suggests that MARK also regulate MT-dependent cellular transport [34]. Overexpression of MARK4 (either S or L isoform) affects MT density, and silencing MARK4S alters centrosomes orientation in G1-arrested cells [35]. Additionally, MARK4L has been also reported to co-localize with vimentin [35]. Par-1b/MARK2 has also been reported to regulate the actin cytoskeleton [21], and a recent study shows the importance of MARK4 in regulating ciliogenesis [22]. Additionally, FEZ1, a kinesin-1 adapter, involved in neuronal transport is regulated by MARK/Par-1 through phosphorylation of a Ser58 regulatory site [36]. These studies clearly indicate to a broader role of MARK in regulating cell cytoskeleton.

Association of MARKs to tauopathy in AD

Although toxic effects of tau are frequently attributed to their aggregation into NFTs, this aggregation is preceded by a number of 'pre-phosphorylation' events involving MARKs and other tau kinases [37, 38]. Figure 2 shows a schematic of the role of MARKs in the events of tau hyperphosphorylation. MARKs are activated following their phosphorylation at Thr208 residue by upstream effector kinases, such as liver serine/threonine kinase B1 (LKB1) and MARKK [33, 39–41]. A recent study further shows that MARK/Par-1 is activated downstream of NMDA receptors in hippocampal neurons through protein kinase A-dependent phosphorylation of LKB1 Ser431 [42]. Activated MARKs phosphorylate tau at Ser262 and make

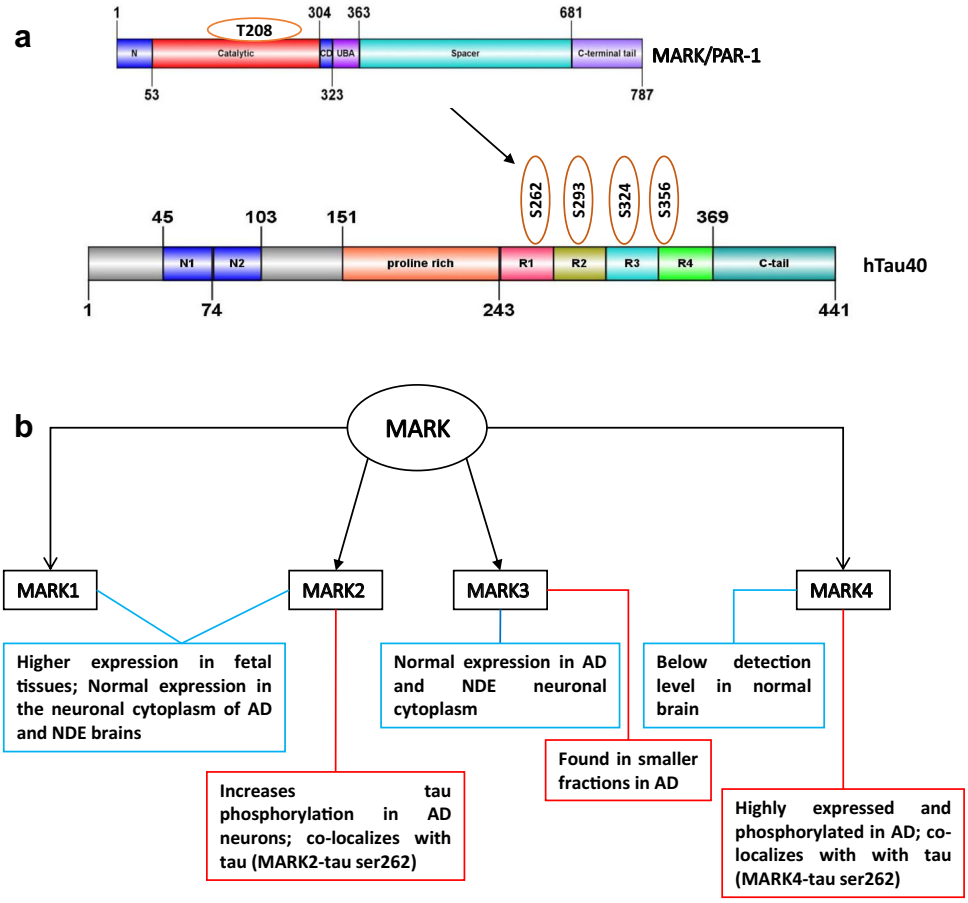

Fig. 1 Full length tau and MARK isoforms expression in AD. **a** Bar diagram showing the structure of full length human tau40 (hTau40) with KXGS motifs (Ser262, Ser293, Ser324, and Ser356) phosphorylated by MARKs. The domain structure of MARK2 phosphorylated by LKB1/MARKK at Thr208 residue is shown. The bar diagrams were generated using IBS 1.0. **b** A summary of expression of different MARK isoforms in AD brain and normal tissue. *NDE* non-demented elderly

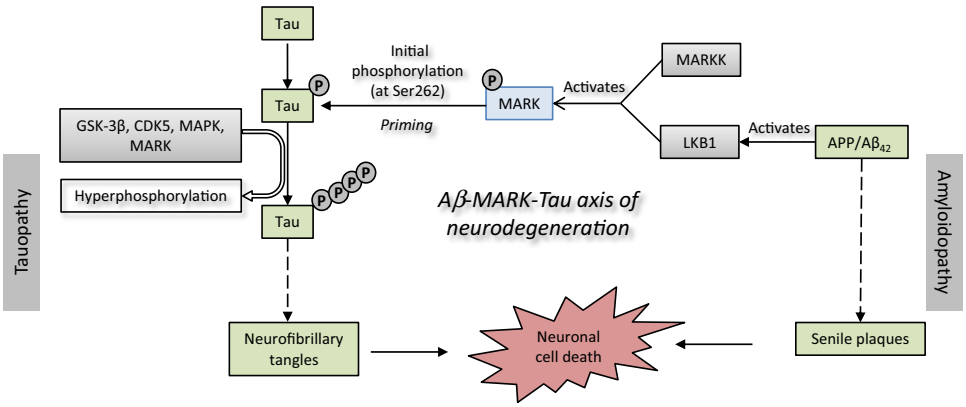

Fig. 2 Aβ-MARK-Tau axis of neurodegeneration. A schematic showing the role played by MARKs in the process of hyperphosphorylation of tau

MT-unbound/bound tau susceptible to hyperphosphorylation by other MAP kinases, such as GSK-3, CDKs, and MAPKs, including MARKs [43, 44]. These events are clearly evident in a study by Ando et al. [45] that shows that MARK/Par-1 initiates the phosphorylation of MT-unbound tau at Ser262/356 in a *Drosophila* model of AD. This 'pre-phosphorylation' stabilizes the less phosphorylated tau, making them more prone to Aβ-mediated processing into toxic tau species. Interestingly, co-expression of amyloid precursor protein (APP) and MARK/Par-1 results in the phosphorylation of Par-1 Thr408 (*Drosophila* Par-1 Thr408 is the same conserved site as Thr208 in MARK2) and tau [46]. This study also provides the first evidence to show how activated MARK/Par-1 is regulated in vivo [46].

Members of MARK3 family (EMK and C-TAK1) have also been reported to phosphorylate Cdc25c phosphatase, PTPH1 tyrosine phosphatase, MAPK-scaffolding KSR1 protein, plakophilin 2, and class IIa histone deacetylases [47–50]. C-TAK1-induced phosphorylation of histone

deacetylases alters their subcellular localization, which is associated with a number of disorders [51, 52]. Additionally, C-TAK1 partially alters the activity of protein phosphatase 2 (PP-2), resulting in the inhibition of GSK-3β-mediated phosphorylation of PP-2 [53]. Defects in PP-2 activity and function are well-reported to cause tau hyperphosphorylation in AD [54]. Intriguingly, MARKs have also been reported to phosphorylate doublecortin, an MAP implicated in X-linked lissencephaly [55].

Given the key role of MARKs, it is not surprising that AD and non-demented elderly (NDE) brains show significant differences in the levels of individual MARK isoforms [16]. Gu et al. report an increased co-localization of phosphorylated tau with MARK2 in AD brains compared to NDE controls [56]. Although at mRNA level, all isoforms of MARK show a uniform neuronal distribution. At the protein level, only MARK1 and MARK2 are evenly distributed in the cytoplasm and neuropils of NDE and AD brains [37]. MARK3 shows only a weak neuronal cytoplasmic staining in AD and NDE brains, in contrast to MARK4, which is largely absent in NDE neuronal cytoplasm [37]. Elevated levels of MARK4 protein and MARK4-tau interaction in AD brains correlate with Braak stages of the disease, directly implicating MARK4 to AD progression [16]. This is also indicated by a progressive accumulation of phosphorylated MARK4 and Ser262-phosphorylated tau in granulovacuolar degeneration bodies (GVDs) of AD brains [16, 37]. In contrast to MARK4, only a subset of GVD-containing neurons show the presence of MARK3 [37].

MARKs in cancers and metabolic disorder

In addition to AD, overexpression of MARKs in different cancer types is associated with metastasis and chemoresistance [26, 27, 57–59]. MARK2 has been associated with cisplatin resistance in lung cancer cell lines [60], and a recent study by Hubaux et al. shows that MARK2 is altered at both DNA and RNA levels in non-small cell lung cancer patient cohorts [58]. Additional studies in lung cancer cell lines suggest that the oncogenic role of MARK2 is not kinase dependent, implicating MARK2 overexpression to cisplatin resistance [58]. The data from cell lines studies also correlate with clinical outcome of cisplatin therapy in lung cancer patients showing high expression of MARK2 [58, 60]. Pardo et al. [61] show that miR-515-5p directly regulates MARK4 activity at post-transcriptional level and results in the inhibition of migration of breast cancer cells. Interestingly, high miR-515-5p and low MARK4 expression levels correlate with increased survival of breast and lung cancer patients [61]. A recent study also shows that knockdown of MARK4 decreases tumorigenic properties of breast cancer MDA-MB-231 cells due to attenuation of MARK4-mediated inhibition of Hippo signaling [62].

In addition to cancers, other studies indicate a potential role of MARKs in metabolic disorders [20, 63, 64]. MARK4 knockout mice show increased metabolic activity and resistance to high fat diet-induced obesity [20, 65]. MARK4 induces oxidative stress and aggravates adipose inflammatory responses by activating the IKKα/NF-κB pathway [65]. However, transcriptional suppression of MARK4 by PPARγ significantly reduces the oxidative stress induced by MARK4.

Rationale to target MARKs for AD

The evidence from various studies clearly present the multifaceted role of MARKs under physiological and diseased conditions [32, 36, 42]. The data from the dual-gene *Drosophila* model indicate that MARKs act as initiators of tau hyperphosphorylation, suggesting their role in the early events of tauopathy [45]. This is also evident from the presence of MARK3 and MARK4 in NFTs of AD brains [16, 66, 67]. Therefore, altering the activity/overexpression of MARKs in AD may prevent MARK-mediated tau toxicity as evident in the study by Chen et al. [32]. Additionally, APP-mediated LKB1-induced activation of MARK/Par-1 suggests that MARKs might be the link that connect Aβ-tau pathologies in AD [46]. Knockdown of endogenous LKB1 prevents APP-mediated phosphorylation of MARK/Par-1 and tau toxicity [46], further suggesting the potential of targeting aberrant MARK activity for preventing tau-related toxicities. Besides, the involvement of MARK4 in tumorigenesis, metastasis, and drug resistance highlights the therapeutic potential of anti-MARK molecules for lung and breast cancer therapy [23, 61, 62]. In the section below, we present an update on currently known inhibitors of MARKs. Although a number of compounds are known to have anti-MARK activity (Fig. 3), their translation into potential anti-MARK drug candidate still lags behind due to under-exploration of these inhibitors.

Small molecule inhibitors of MARKs

Staurosporine

Staurosporine (STS) is a pan-kinase inhibitor that induces apoptosis and G_1 cell cycle arrest in various cell types [68]. In addition to its effect on protein kinases C, A and G, STS alters the expression of cyclin-dependent kinase inhibitors, and leucine-rich repeat kinase 2 associated with Parkinson's disease at nanomolar range [69]. STS treatment

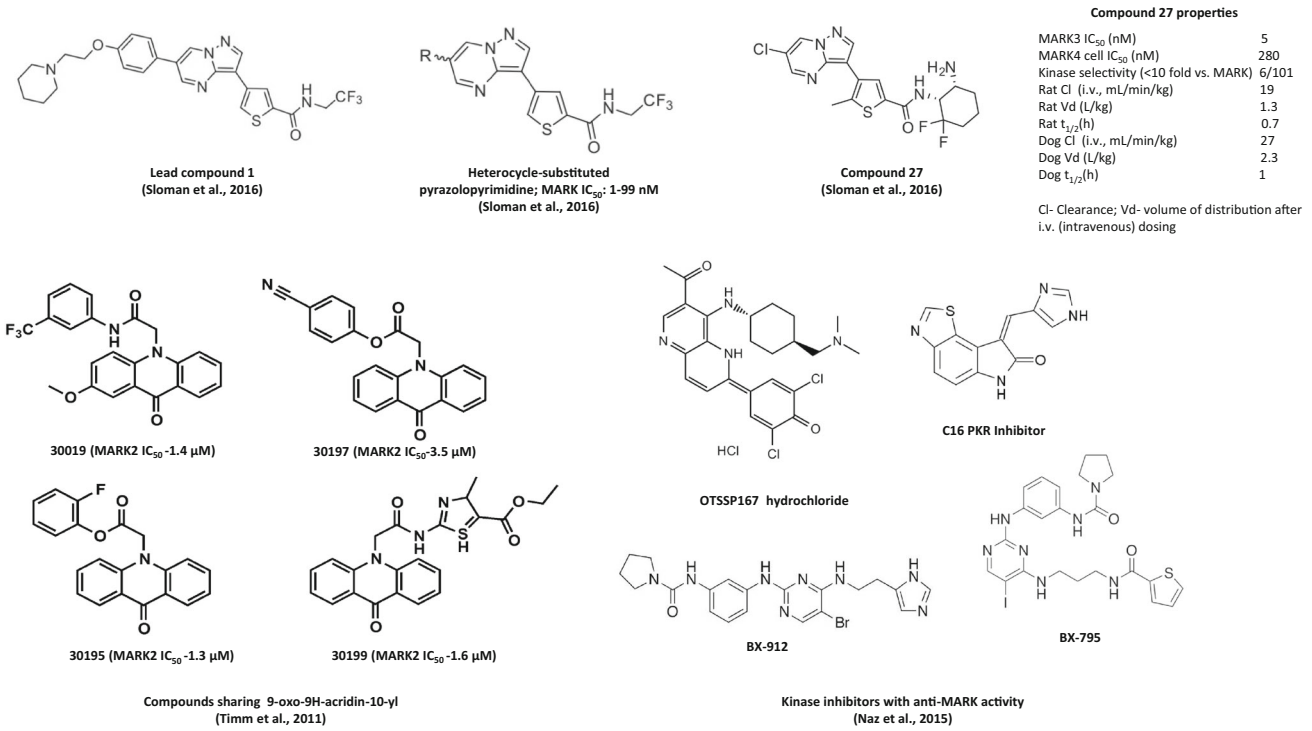

Fig. 3 Structures of MARK inhibitors. Chemical structures of MARK inhibitors from Sloman et al. [102], Timm et al. [88], and Naz et al. [96] are presented. The MARK half-maximal inhibitory concentrations (IC_{50}) of compounds are shown next to each structure

results in neurite outgrowth in dopaminergic neuron [70] and elevates choline acetyltransferase activity following basal forebrain lesion in cholinergic neurons [71]. In addition to its neurotropic effects in a number of cell types [70], STS has been reported to attenuate impaired learning in rats [71]. Recently, STS was shown to inhibit MARK-tau interaction, reducing tau phosphorylation at Ser262 in mouse embryonic 3T3 fibroblast cells [16, 56]. Moreover, there was no effect of STS on MARK2 and tau protein levels [56]. However, dose-limiting toxicities and poor pharmacokinetics (PK) [72–75] make STS a less likely choice for development as an anti-MARK therapeutic agent.

Interestingly, STS analog 7-hydroxystaurosporine (UCN-01) shows better anti-tumor effects in animal models of epidermoid carcinoma, fibrosarcoma, and acute myeloid leukemia over STS [74]. UCN-01 is currently under clinical trials for cancer therapy, in addition to another analog, *N*-benzoyl-staurosporine (PKC412) [76]. See Monnerat et al. [76] and Sausville et al. [77] for detailed PK properties of PKC412 and UCN-01, respectively. In addition to analogs, liposomal encapsulation has been reported to enhance the anti-tumor activity of STS in mice without resulting in adverse effects [78]. Moreover, liposomal encapsulation reduces the circulation half-life of STS in comparison to free STS [75, 78]. These properties make STS analogs and liposomal formulation promising compounds for further exploration in relation to MARKs. Although currently there are no data on anti-MARK effects of liposomal STS, a single study in a cell-free system reports the anti-MARK3 activity of UCN-01 [79].

Methylene blue

The therapeutic effects of methylene blue (MB), a phenothiazine dye, against a number of diseases including AD are well-reported in the literature [80–82]. MB permeates the blood–brain barrier (BBB) following either intravenous, intraperitoneal, or intraduodenal dosing; however, the level of MB uptake is higher in the CNS when administrated intravenously [83]. Although MB is known to target multiple AD-specific molecular targets [84], a recent study reported the first anti-MARK4 effects of MB in a fly model of MARK4/Par-1 overexpression [85]. MB decreased Par-1 protein levels at neuromuscular junctions and rescued synaptic loss without affecting the expression of human leucine-rich repeat serine/threonine-protein kinase 2, suggesting MB specifically targets MARK4/Par-1. Similarly, MB also downregulated MARK4-induced tau Ser262 phosphorylation in a cell-free assay and 293T cells expressing human 4R2N tau in a dose-dependent manner. The study also shows that MB inhibits tau phosphorylation at Ser396 and Ser214 in 293T cells, presumably due to MB effect on GSK-3β and

CDK5. MB decreased MARK4 protein level plausibly by proteasomal degradation of ubiquitinated MARK4, suggesting that the ubiquitin–proteasome pathway is also a potential target of MB [85]. Since MB has been reported to induce autophagy and decrease tau protein levels [86], it is also likely that the observed effect of MB on MARK4 may have been partly due to the induction of autophagy [85].

Proline-directed hymenialdisine

The marine natural product hymenialdisine (HD01) was reported to competitively inhibit proline-directed kinases, such as GSK-3β- and CDK, and tau hyperphosphorylation at AD-specific sites [87, 88]. Additionally, HD01 inhibits non-proline-directed MARK2 at nanomolar concentration by binding at the catalytic pocket of MARK [31]. However, since the discovery of MARK inhibitory effects of HD01, there has been no progress in the development of HD01 as an anti-MARK agent. Although a number of HD01 analogs were synthesized [89], none of these compounds were reported to have any anti-MARK activity. Two other proline-directed potent GSK-3β inhibitors, SB-216763 and SD-415286, are known to interact with MARK3 and its activating kinase, MARKK [79, 88]. Although there are no additional data on anti-MARK activity, both SB-216763 and SD-415286 are known to have neuroprotective effects [90].

9-OXO-9H-acridin-10-yl compounds

The Mendelkow group at the Max-Planck-Unit for Structural Molecular Biology in Hamburg, Germany, reported the identification of four low-molecular weight anti-MARK2 compounds (30019, 30195, 30197, 30199) sharing a common 9-OXO-9H-acridin-10-yl functional group (Fig. 3) [88]. The compound 30019 inhibited all MARK isoforms in a dose-dependent manner. Further, the compounds were not effective against GSK-3β and showed little to no cross-reactivity against close relatives of MARK, such as SAD kinase B and AMP-activated protein kinase (AMPK). Abolition of MT disruption in CHO cells overexpressing MARK2 following treatment with the compounds suggests that this class of compounds possibly inhibit MARK2-mediated hyperphosphorylation of tau [88]. Interestingly, a recent study also highlights the specificity of 9-OXO-9H-acridin-10-yl derivatives for MARK4 and their presumable development into potent anti-prostate cancer candidates [23]. This provides a strong rationale for further exploration of 9-OXO-9H-acridin-10-yl derivatives given the role of MARKs in AD and cancers.

CagA peptide

A toxin of *Helicobacter pylori*, CagA, was shown to inhibit the activity of MARK/Par-1 and disrupt cell polarity in canine kidney MDCK cells [91]. CagA is also reported to affect a number of other kinases involved in different signaling pathways, including protein kinase C-related kinase 2 (PRK2) [91, 92]. CagA lacking the Par-1/MARK-binding domain continues to inhibit PRK2, suggesting that CagA interacts with PRK2 and Par-1/MARK via two different domains [92]. This is evident from the study of Neišić et al. that shows that a sub-domain of CagA containing a short chain of 14 amino acid occupies the substrate binding site of MARK2 [93]. A mutation in the amino acid sequence abolishes CagA interaction with MARK2. This peptide of CagA—termed as MARK2 kinase inhibitor (MKI)—inhibits basal kinase and MARKK-activated activity of MARK2 at micromolar range [93]. MKI dramatically inhibits MARK4 activity in rat hippocampal neurons, reducing tau hyperphosphorylation at Ser262 residue [28]. Additionally, MKI-mediated inhibition of MARK4 activity abrogates Aβ-mediated loss of dendritic spines and synapses in neurons [32]. The lack of effect of MKI on acetyl-CoA carboxylase phosphorylation by AMPK suggests that MKI specifically targets MARK/Par-1 [32]. Although the above studies provide encouraging evidence to develop MKI as an anti-MARK agent [28, 93], whether MKI will have any therapeutic benefits in MARK-directed AD therapy requires extensive studies.

Other known protein kinase inhibitors with anti-MARK effects

C16 is a known neuroprotective protein kinase R (PKR) inhibitor that prevents PKR-induced neuronal death and inflammatory cytokines release in animal models of brain injury [94, 95]. Additionally, C16 prevents nuclear translocation of Fas-associated protein with a death domain and Aβ-induced apoptosis in human neuroblastoma SH-SY5Y cells [96]. Intraperitoneal administration of C16 results in dose-dependent decrease in PKR phosphorylation in rat brain, indicating the ability of C16 to penetrate the BBB [95]. A recent study reports the promising effects of C16 as a lead anti-MARK4 candidate [92]. This inhibitory effect results from the interaction of C16 with the hydrophobic cavity of MARK4 [97].

Three other kinase inhibitors (BX-912, BX-795, and OTSSP167) already known to have anti-cancer effects were also reported to have anti-MARK activity (Fig. 3). The inhibitors have a similar binding pattern to MARK4 kinase domain [98]; however, OTSSP167 forms a more stable complex with MARK4 [98]. Not surprisingly,

OTSSP167 results in better inhibition of MARK4 compared to BX-912, BX-795, and C16 [99]. A similar inhibitory effect of BX-795 was previously reported against MARK3 [79]. BX-795 and BX-919 are known inhibitors of 3-phosphoinositide-dependent kinase-1/AKT signaling [100], whereas, BX-795 also affects kinases that are involved in immune responses [79, 100, 101]. OTSSP167 inhibits maternal embryonic leucine zipper kinase overexpressed in a number of aggressive tumors presumably explaining the potent anti-cancer effects in different tumor cell types and xenograft models [102]. Although these data provide a new insight into anti-MARK4 activity of BX-912, BX-795, OTSSP167, and C16 [98], additional studies are necessary to advance these compounds as anti-AD candidates. Since these agents are already known to have anti-cancer effects, their potential use for reversing MARK-induced drug resistance in cancers will benefit cancer therapy.

Pyrazolopyrimidines are protein tyrosine kinase inhibitors with potent anti-tumor effects [103, 104]. Recently, Sloman et al. reported the preferential anti-MARK activity of a pyrazolopyrimidine inhibitor [105]. Furthermore, the derivative was reported to have better PK and cell anti-proliferative properties than the parental compound (see Fig. 3 for biochemical and cellular properties of this compound). A recent co-crystal structure study revealed that pyrazolopyrimidine interacts with MARK4 at the ATP site and in close proximity to a position that potentially alters the catalytic loop of MARK4 [106]. This is likely the reason for the potent MARK inhibitory effects of pyrazolopyrimidines [105, 106]. Given the improved PK properties and CNS penetration of the pyrazolopyrimidine derivative [105], additional studies in cell and animal models of AD are required to show the potential therapeutic benefits of pyrazolopyrimidines.

Targeting MARKs via indirect pathways: a perspective

The activity of MARK is regulated by activating kinases and interaction with scaffolding and other cellular proteins, making them potential indirect targets for altering the activity of MARKs [107, 108]. LKB1 regulates MARK activity by phosphorylating Thr residue at activation loop [40, 46]. Knockdown of LKB1 in mouse embryonic fibroblasts markedly reduces the catalytic activity of MARK1–4 [40]. In contrast, ectopic expression of LKB1 increases MARK4 phosphorylation [33]. Likewise, synthetic LKB1 peptides also alleviate the activity of all MARK types. Aβ-mediated increase in MARK activity occurs in a LKB1-dependent manner and contributes to tauopathy in AD [46]. These data indicate that LKB1 is a master regulator of MARK and interventions that can modulate LKB1 activity/expression may alter MARK-induced tau hyperphosphorylation [109]. Although LKB1 signaling is being investigated as druggable target, and LKB1 knockout has been reported to reduce MARK activity [40], a number of studies also show the deleterious effects of targeting LKB1 [110, 111]. This is not surprising given the role of LKB1 in several vital physiological functions.

Heterodimerization of MARK2 with protein kinase PAK5 results in the inhibition of MARK activity, indicating dimerization as another potential indirect mechanism for targeting MARK activity. This is in fact evident from the study of Sun et al. that suggests that MB-induced inhibition of MARK4 activity presumably results from the stabilization of covalently dimerized MARK4 [85]. The other mechanisms that are known to inhibit MARK activity are polyubiquitination and binding with scaffold protein 14-3-3 [112–114]. Both MARK2 and MARK3 are negatively regulated by aPKC [63], and MARK4 that was previously known to interact with aPKC [33], also undergoes aPKC-mediated phosphorylation at serine and threonine sites [115]. In addition to aPKC, Watkins et al. show the regulation of Par-1b/MARK2 activity by novel protein kinase C (PKC) and protein kinase D [116]. This is further confirmed in cells following treatment with PKC-activating agent phorbol-12-myristate-13-acetate which results in phosphorylation of Par-1b/MARK2 at Ser400 [116]. These studies clearly show that members of PKC family regulate MARKs, and modulating their functions may indirectly alter MARK activity.

In addition to tau, GSK-3β also phosphorylates MARK at Ser212 and results in the inhibition of its kinase activity under in vitro condition [117]. Interestingly, there is a marked change in the pattern of tau phosphorylation in AD following a concomitant activation of GSK-3β and inactivation of MARK [117]. In contrast to Timm et al. [117], Kosuga et al. show that phosphorylation of MARK2/Par-1 at Ser212 activates MARK2, resulting in tau phosphorylation at Ser262 [118]. However, co-expression of cells with wild-type and constitutively active mutants of GSK-3β and MARK2 reduces tau phosphorylation, confirming that GSK-3β rather inhibits the activation of MARK2 [117]. The aPKC/GSK-3β and MARK signaling cascades present another potential window for exploring methods that can indirectly alter MARK activity under pathological conditions.

Conclusion

Although AD is a multifactorial disorder, the precise mechanism that contributes to the development and progression of the disease remains ambiguous [119]. Studies now clearly

indicate that the members of MARK family are clearly associated with cancers and AD in humans [20, 27, 37]. Moreover, it is also evident that MARKs have a larger role in the pathological events of AD [19, 45]. Therefore, development of specific inhibitors of MARKs will not only serve as promising therapeutic candidates, but also broaden our understanding of the role of each isoform in disease and health. In this context, the recently reported ligand-bound crystallographic structure of MARK4 could potentially serve as a scaffold for the rational design of MARK inhibitors [106]. Besides, pathways that are known to regulate MARK activity may serve as potential indirect strategies for targeting MARKs. However, given the myriad role of MARKs and MARK-regulating kinases, such as LKB1, further studies are essential to validate the implications of these strategies in relation to physiological functions. This is particularly evident from the study of Yu et al. [28]. Although MKI restored neurotransmission in diseased neurons, the decrease in excitatory potential in healthy neurons suggests that maintaining MARK activity at a physiological level is essential for normal neurotransmission.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interests. All authors read and approved the final manuscript.

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