sFRP-mediated Wnt sequestration as a potential therapeutic target for Alzheimer's disease

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Abstract

The extracellular ligand, Wnt, and its receptors are involved in signal transduction and play an important role in axis formation and neural development. In neurodegenerative disorders such as Alzheimer's disease (AD), a decrease of the intracellular Wnt effector, β-catenin, has been linked to amyloid-β-peptide-induced neurotoxicity. Despite this knowledge, targeting Wnt inhibitors as potential biomarkers has not been explored, and harnessing Wnt activators as therapeutic candidates remains largely not investigated. A wide acting family of Wnt mediators, secreted frizzled-related proteins (sFRPs), has not been probed so far as molecular indicators of disease occurrence and progression of Alzheimer's. Unlike the effect of the Dickkopf (DKK) family of Wnt antagonists on AD, the sFRP molecules have a more pleiotropic impact on the Wnt signaling cascade and probably have a far-reaching involvement in neurodegeneration. The role of sFRPs has been poorly described in AD, and in this review, we analyze the present status of the role of sFRPs on neurodegeneration, their likely involvement, and potential implications in treatment modalities of AD. This information would provide valuable clues for the development of potential therapeutic targets for aberrant neurodegenerative disorders.

Keywords

Alzheimer's disease, Wnt, sFRP, neurodegeneration

Introduction

Neurodegenerative diseases represent a diverse spectrum of neuronal disorders with progressive dysfunctions in the nervous system. Pathologically, they are identified by the presence of disease-specific protein aggregations and misfoldings in specific regions of the brain (Jucker & Walker, 2013). Although several neurological disorders have been identified, attention has been given to only a handful, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, and amyotrophic lateral sclerosis (Ross & Poirier, 2004). The prevalence and incidence of these diseases increase with age (Melo et al., 2011), and reaches a peak well before the maximum lifespan. For instance, AD is the 5th leading cause of death globally for those aged 65 and older. An increase in incidence of 40% of AD has been reported worldwide and is predicted to rise dramatically to one new case diagnosed every 33 seconds, which is projected to lead to around one million new cases per year by 2050 (http://www.alz.org/facts/).

Clinically, AD is characterized by the gradual decline of neuro-cognitive functions (Gouras et al., 2010). The hallmark identification of β-amyloid (Aβ) deposits and neuro-fibrillary tangles (NFTs), formed by hyperphosphorylated tau protein in the sub-cortical regions of the brain (as reported in several studies Armstrong, 2011; Ittner and Götz, 2011; Blennow et al., 2006)), correlates with the synaptic dysfunctions and massive neuronal death observed in AD (Fuentealba et al., 2004). The Aβ deposition also results in the activation of pro-inflammatory cytokines primarily in the glial cells (Lindberg et al., 2005), initiation of the complement cascade (Aisen, 1997), and the induction of pro-inflammatory enzymes such as inducible nitric oxide synthase and cyclooxygenase-2; these factors further contribute to neuronal dysfunction and cell death (Brown and Bal-Price, 2003). The key focus of current AD research is on the underlying disease mechanisms, and a number of studies have

been conducted globally to identify the potential biomarkers for this disease.

Wnt signaling in AD

Although the underlying molecular map of AD remains elusive, impaired Wnt signaling events are well known to promote AD pathogenesis (Inestrosa and Toledo, 2008). Wnts (Wingless) are a family of 19 members of secreted lipoglycoproteins that are multifunctional in nature (Willert and Nusse, 2012) . The Wnt pathways are crucial in cell fate determination during animal development (Martin and Kimelman, 2012) and are known to regulate many developmental events in the nervous system, including the maintenance of synapses during brain development (van Amerongen and Nusse, 2009; Inestrosa and Varela- Nallar, 2014) and also in dorsal-ventral patterning in the forebrain (Harrison-Uy and Pleasure, 2012; Quinlan et al., 2009). Furthermore, ablation of Wnt variants in neuro-degenerative diseases has been reported to result in severe midbrain damage and hippocampus dysfunction (Arenas, 2014; Varela-Nallar and Inestrosa, 2013). Different mechanisms of Wnt signaling have been identified; namely, the ß-catenin dependent and ß-catenin independent pathways. The Wnt/ß-catenin pathway has been most extensively studied in neurodegeneration, especially during AD progression (Wan et al., 2014; Godoy et al., 2014). In this pathway, in the presence of Wnt ligands, signals are transduced to the nucleus via ß-catenin through the interaction of Wnt with a 7-transmembrane receptor complex, the frizzled receptor (FZD), and a co-receptor corresponding to the low density lipoprotein receptor-related protein 5/6 (LRP 5/6) membrane protein, which leads to the activation of dishevelled (DVL) (MacDonald and He, 2012; Esteve et al., 2011).

These events stabilize intracellular ß-catenin through the axin complex, which is composed of axin, the tumor suppressor adenomatous polyposis coli gene product (APC), and glycogen

synthase kinase 3 beta (GSK3ß), thereby preventing its phosphorylation and ubiquitin mediated degradation (Fig 1). It has been reported that, in response to depolarization, the maintenance of intracellular ß-catenin levels is crucial in regulating neuronal homeostasis and synaptic modulations (Murase et al., 2002). Depleted levels of ß-catenin have been correlated with synaptic loss and Aβ deposition (Chen and Bodles, 2007), and have been identified as key factors prior to the onset of neuronal death in many neurodegenerative diseases including AD. The ß-catenin-independent pathway of Wnt signaling has two arms: the Wnt/planar cell polarity (PCP) pathway and the Wnt/Ca^{2+} signaling pathway. In the Wnt/Ca^{2+} pathway, Wnts binds to FZD resulting in stimulation of heterotrimeric G proteins, which further activates phospholipase C. This leads to increased Ca^{2+} release and activates two kinases: Ca^{2+}-calmodulin dependent protein kinase-II and protein kinase-C. This, in turn, stimulates transcription factors such as cAMP response element-binding protein-1. In the PCP pathway, Wnt proteins bind to FZD, which activates small GTP-binding proteins Rho and Rac and jun N-terminal kinase via DVL. This interaction results in cytoskeletal regulation and involves polarized cell shape changes and migration (Fig 1).

Wnt/ß-catenin signaling plays a crucial role in embryonic development of most tissues and organs of the body (van Amerongen and Nusse, 2009), and it is also important in the adult organisms, where it maintains hair, skin (Alonso and Fuchs, 2003), intestinal health, and hematopoiesis (Malhotra and Kincade, 2009). Aberrant regulation of ß-catenin levels and the ß-catenin signaling pathway have been associated with the onset of fibrosis and metabolic disease, as well as many cancers including lung, pancreas, breast, and colorectal cancers (Paul and Dey, 2008; Kahn, 2014). The Wnt/ß-catenin dependent pathway has also been extensively studied in neurodegenerative diseases, especially with respect to its involvement in AD progression. For example, studies on ß-catenin in neuronal cells have reported that neural depolarization induces ß-catenin redistribution from dendritic shafts into spines, where

it interacts with cadherin to influence synaptic size and strength (Murase et al., 2002); thus, the maintenance of intracellular ß-catenin levels is crucial in the regulation of neuronal homeostasis and synaptic modulations. It has also been shown that depletion of ß-catenin levels correlates with synaptic loss and Aβ deposition (Inestrosa and Arenas, 2010).Furthermore, a deficiency in LRP6-mediated Wnt signaling in an AD transgenic mouse model led to greater amyloid deposition due to greater Aβ production; and deletion of the LRP6 gene in mice forebrain neurons led to age-dependent deficits in synapse integrity as well as memory deficits (Liu et al., 2014).

Many studies have investigated the relationship between components of the Wnt/ß-catenin signaling pathway and AD-related proteins. For example, the amyloid precursor protein (APP) intracellular domain (AICD), which is present in higher levels in AD, has been shown to bind to GSK3β, activating it and thereby reducing β-catenin levels. Another very pertinent observation showing a direct link between amyloid-β and Wnt signaling indicated that amyloid-β binds to the FZD cysteine-rich domain in close proximity to the Wnt-binding site and inhibits the Wnt β-catenin signaling pathway (Magdesian et al., 2008). In AICD-transgenic mice (Ryan and Pimplikar, 2005), studies found hyper-phosphorylation and aggregation of tau, neurodegeneration, and deficits in working memory that could be prevented by treatment with lithium chloride, a GSK3β inhibitor (Ghosal et al., 2009). However, the AICD-Wnt pathway relationship needs further study, as AICD has also been shown to bind to β-catenin (Zhou et al., 2011), and some neuronal studies have found GSK3β activation causes neurite retraction (Sanchez et al., 2001). AICD-stimulated GSK3β has resulted in neurite outgrowth in the PC12 and N2A neuronal cell lines as well as in primary neurons (Zhou et al., 2011). The AICD pathway also should be investigated further as it appears to be able to contribute to AD pathology independently of Aβ. Another controversial link between AICD and AD involves neprilysin (NEP), a major Aβ-degrading enzyme;

although some previous studies have not agreed with these findings, a recent comprehensive study has found NEP expression to be dependent on AICD levels in several transgenic AD mouse models as well as in cell culture models (Grimm et al., 2015). In recent years, GSK3β, being a key modulator of the Wnt β-catenin pathway, has emerged as one of the most relevant targets for AD treatment (Martinez and Perez, 2008). Lithium chloride (LiCl), a classic inhibitor of GSK3β, has been shown to prevent neurotoxicity by reducing amyloid-β production in a mouse model of AD (Phiel et al., 2003). LiCl has also been reported to inhibit amyloid-ß induced synaptic degeneration in elderly AD patients with bipolar disorders (Valvezan and Klein, 2012). An increasing number of novel GSK3β inhibitors, which are both ATP-competitive and non-ATP-competitive, have been developed; particularly promising are the non-ATP-competitive GSK3β inhibitors, since they are more selective and less toxic (King et al., 2014), examples of which being L803-mts (Plotkin et al., 2003; Kaidanovich-Beilin et al., 2004) and VP0.7 (Palomo et al., 2011). The typical ATP-competitive GSK3 inhibitors include indirubin (Leclerc et al., 2001), paullone compounds (Leost et al., 2000), SB415286 and SB216763 (Coghlan et al., 2000), and AR-A014418 (Bhat et al., 2003). Similarly, it has been shown that bromoindirubin-30-oxime (6-BIO), another GSK3ß inhibitor, was able to reduce amyloid-ß formation and prevent neuronal apoptosis (Silva-Alvarez et al., 2013).

Wnt signaling has also been shown to influence levels of β-amyloid precursor protein cleaving enzyme-1 (BACE1), as chromatin analysis has demonstrated that β-catenin binds specifically to regions within the BACE1 promoter that contain putative T-cell factor/lymphoid enhancer factor (TCF/LEF) motifs, consistent with canonical Wnt target regulation (Parr et al., 2015). Furthermore, TCF4 was found to act as a transcriptional repressor of *BACE1*. All these results support the concept that the modulation of Wnt/β-catenin signaling may be a pathway to potential preventative treatments for AD.

Studies of Wnt signaling and mitochondria have found that β-catenin dependent Wnt signaling (via the Wnt3a ligand) prevents the permeabilization of mitochondrial membranes, induced by Aβ oligomers, through the inhibition of the mitochondrial permeability transition pore. It has also been found that β-catenin independent Wnt signaling, through the Wnt5a ligand, protects mitochondria from mitochondrial fission-fusion alterations that occur in AD, and also modulates B-cell lymphoma 2 (Bcl2) increases, which are induced by Aβ oligomers (Arrázola et al., 2015; Silva-Alvarez et al., 2013). These findings suggest that modulation of Wnt signaling (both β-catenin dependent and independent) could also protect against AD-related mitochondrial dysfunction. Another emerging line of evidence links Wnt signaling with the inflammatory response in microglia, which is central to neuroinflammation in neurodegenerative diseases. Disease associated β-catenin accumulation was shown in microglia *in vivo* in mice with Alzheimer's-like pathology (Schnurrbusch et al., 1990). The Wnts were also shown to differentially activate β-catenin dependent and independent signaling in a murine microglia cell line, and are possible regulators of microglia-mediated neuroinflammation (Kilander et al., 2011).

Wnt antagonists as key modulators in AD cell signaling

Antagonists of Wnt have been shown to work in tandem with Wnt activators during development to promote or inhibit a variety of developmental pathways. They are reported to act either intracellularly by affecting signal transduction, or extracellularly by altering their ability to bind the membrane-receptor complex (Cruciat and Niehrs, 2013). The inhibitors of Wnt are classically grouped into two functional classes: the Dickkopf (Dkk) group of Wnt antagonists comprised of the Dkk family 1-5, which regulate Wnt signaling by binding to the Frizzled co- receptor component LRP5/LRP6; and the secreted frizzled-related protein (sFRP) group, which binds directly either to Wnt or its receptor, FZD, and modulates the

downstream signals (Kawano and Kypta, 2003). The role of Dkk-1 in response to Wnt signaling, and its antagonistic properties, has been studied extensively by several groups**.** Higher expression of Dkk-1 has been associated with cell death and DNA damage (Slee et al., 2004), while the expression level of Dkk-1 has been shown to be low in the normal healthy adult brain (Galli et al., 2014; Scott and Brann, 2013). Early reports from studies of cultured cortical neurons describe a potential role of Dkk-1 in the induction of Aß toxicity-mediated inhibition of Wnt cascades through interaction with LRP5/6 (Caraci et al., 2008; Caricasole et al., 2004). In parallel, significantly increased expression of Dkk-1 has also been detected in *post-mortem* AD brains and transgenic mouse AD models (Lieven et al., 2010; Mukhopadhyay et al., 2001); in fact, in one AD mouse model, Dkk-1 has been found to colocalize with hyper-phosphorylated tau-bearing neurons and active GSK3β (Rosi et al., 2010).

Furthermore, in studies of cultured neuronal cells that had high levels of Dkk-1 due to incubation with Aβ peptides, it was found that antisense knockdown of Dkk-1 resulted in significant inhibition of the Aβ-induced hyper-phosphorylation of tau protein (Caricasole et al., 2004). Recent studies of SAMP8 (senescence-accelerated) mice detected higher than normal levels of Dkk-1, activated GSK3β, and hyper-phosphorylated tau, along with higher than normal levels of β-catenin that had been phosphorylated and ready for degradation; and lower protein levels of the anti-apoptotic protein Bcl2, a Wnt target gene (Itasaki et al., 2003). Together, all these data suggest that Dkk-1 plays a central role in the pathological cascades of AD.

In the ß-catenin Wnt pathway, accumulation of ß-catenin has been shown to be inhibited by the active binding of Wise/SOST (Sclerostin) antagonist family members through their interaction with either Wnt-1 or Wnt-3a in *Xenopus* animal cap cells (Bayod et al., 2015). A

similar observation was reported with other Wnt antagonists such as Wnt inhibitory factor (WIF)-1, which is expressed in the nervous system (Bayod et al., 2015) where it mediates ß-catenin degradation by direct interaction with Wnt ligands. Although there are reports on the Wnt inhibitors acting through the LRP pathway, little is known about the inhibitors acting through the Frizzled receptor, namely the sFRPs, and their role as Wnt inhibitors in neurodegenerative diseases. Here, we review the recent developments in sFRP-Wnt interactions and the emerging role of sFRPs as key Wnt antagonists in AD neuropathology.

sFRPs as multifunctional regulators

sFRPs are known to bind directly to Wnt, and have sequence and structural similarities with FZD proteins (Rattner et al., 1997; Finch et al., 1997). The sFRP family is comprised of 5 members in which sFRP1, 2, and 5 are clustered together as a group based on their sequence similarities, and are distantly related to the other cluster comprising sFRP3 and sFRP4 (Garcia-Hoyos et al., 2004). Initial studies on sFRPs in *Xenopus* embryos (Leyns et al., 1997) designated them as Wnt antagonists by their ability to bind to Wnt and block Wnt/ß-catenin signaling. The role of sFRP was further demonstrated in Drosophila (Lin and Perrimon, 1999; Uren et al., 2000) by their ability to inhibit the activity of wingless-ligand Wg, a homologue of Wnt. Since this discovery, the functions of sFRPs have been progressively unravelled to demonstrate that they have multifunctional roles in regulating Wnt signaling in development and disease (Surana et al., 2014). The binding affinity of sFRPs has been shown to vary between their Wnt partners (Galli et al., 2014). and the sFRPs are reported to initiate complex formation with FZD proteins (Kawano and Kypta, 2003; Misra and Matise, 2010), indicating that the molecule could interact not only with Wnt but also with FZD receptors. sFRPs have two independent domains: the N-terminus consisting of a secretory signal peptide and a cysteine-rich domain (CRD), which is identical to the putative Wnt binding site of FZD receptors; and the C-terminus end, which has heparin binding residues and a netrin-related

motif (NTR) (Dann et al., 2001). The overall functions of the NTR are not defined, yet there are studies reporting its involvement in Wnt binding (Lopez- Rios et al., 2008) and in antagonizing tube formation and promoting apoptosis of human umbilical vein endothelial cells (Longman et al., 2012). Despite the conflicting reports on these two domains in potentiating Wnt activities (Dann et al., 2001; Bovolenta et al., 2008), a parallel study by Bhat et al, has demonstrated the fundamental importance of both domains in modulating Wnt dependent and independent signaling cascades (Bhat et al., 2007). However, due to the sequence resemblance of the CRD with FZD proteins, the CRDs of sFRPs are more recently postulated to be crucial for inhibiting Wnt binding to FZD (Scott and Brann, 2013; Martin-Manso et al., 2011). The ability of sFRPs to bind FZD via their CRD by forming homo- and heterodimers can also stimulate signal transduction (Carron et al., 2003). The biphasic effect of sFRPs, mainly via their CRD region, is dependent on their concentration and the cellular context. sFRP1 and 2 have been shown to either increase or decrease β-catenin stabilization depending on the cell type and the expression pattern of the FZD receptors (Xavier et al., 2014). The extracellular stoichiometry of sFRPs, Wnt, and FZD, and the intricate interplay of these molecules with each other is probably a deciding factor in determining whether the balance swings to sFRPs being potentiators or inhibitors of Wnt signaling.

sFRPs can function as signal coordinators when multiple cascades are operating in order to determine cell fate and development. For instance, the role of sFRP1 in regulating neural cell proliferation during development has been demonstrated by Augustine and co-workers (Augustine et al., 2001), where they detected a strong expression of *sFRP-1* in the developing neocortex of the mouse during the entire period when neurons for the neocortex were being generated and allocated to their final resting positions. sFRP1 has been implicated in

angiogenesis (Dufourcq et al., 2002), retinal cell differentiation (Kiefer et al., 2013) and axon guidance (Drescher, 2005; Rodriguez et al., 2005). A study by Rodriguez et al. [25] reported the possibility of inactivated sFRP1 and 2 being able to perturb retinal neurogenesis in the mouse embryo. A concentration dependent functional role of sFRP1 and 2 in promoting dopaminergic neuron differentiation has also been demonstrated (Kele et al., 2012). Concurrently, a deletion study with sFRP3 alone reported its implication in the neurogenesis of adult hippocampal cells (Jang et al., 2013).

The pro-apoptotic role of sFRP4 has been well established, initially by Dharmarajan and co-workers, in reproductive tissues such as the mammary gland (Constantinou et al., 2008), ovary (Drake et al., 2003), corpus luteum (Guo et al., 1998) and the uterus (Hewitt et al., 2006). However, the apoptotic function of sFRP4 has been rendered inactive in some cancers (He et al., 2005; Brebi et al., 2014). For example, in ovarian cancer, loss of sFRP4 expression results in an aggressive phenotype with poor prognosis (Jacob et al., 2012; Saran et al., 2012). The role of sFRP4 in glioblastoma and head and neck cancers has been recently reported from our group (Warrier et al., 2013; Warrier et al., 2014). We have shown that sFRP4 clearly sensitizes the cancer stem cells to chemotherapeutics by inhibiting the Wnt/ß-catenin pathway and initiating the apoptotic cascade. Additionally, sFRP4 was seen to undergo hypermethylation at the promoter region in several glioma cell lines (Schiefer et al., 2014).Furthermore, we have recently demonstrated the role of sFRP4 in inhibiting the stemness of glioma stem cells and inducing apoptosis (Bhuvanalakshmi et al., 2015). sFRP4 also blocked other typical cancer stem cells traits such as epithelial to mesenchymal transition in glioma stem cells (Bhuvanalakshmi et al., 2015). These studies validate the hypothesis that the downregulation of sFRP4 facilitates cancer stem cell proliferation in brain tumors.

Beyond their role as an inhibitor, sFRPs stabilize Wnts and regulate both Wnt and bone

morphogenetic protein (BMP) signals for maintaining neural tube functions in vertebrates (Misra and Matise, 2010; Stuckenholz et al., 2013). Furthermore, sFRPs facilitate the diffusion of Wnt ligands extracellularly, thus providing an alternate function to sFRP molecules as Wnt transporters (Mii and Taira, 2009). Altogether, considering these diverse observations on the sFRPs' activity, it can be postulated that they could have a distinct pattern of expression and, depending on the cellular context, sFRPs can either potentiate or regulate several developmental decisions.

Role of sFRP-Wnt interactions in AD development and progression

Although their detailed mechanism of action and their role in neurodegenerative diseases are poorly understood, sFRP-Wnt interactions could orchestrate a series of sequential events for Alzheimer's progression. Being a member of extracellular Wnt inhibitors, sFRPs function similar to other antagonists, such as Dkk-1 (Inestrosa and Toledo, 2008; Caraci et al., 2008) and WIF -1 (Hu et al., 2008), to modulate the Wnt β-catenin dependent pathway. Loss or impairment in Wnt signals has been recently marked as a molecular tag in AD pathogenesis (Inestrosa and Varela-Nallar, 2014). Induction of Dkk-1, and the resultant ß-amyloid toxicity observed in cultured neural cells (Killick et al., 2014; Purro et al., 2014), has been found to be under the mechanistic regulation of a key enzyme modulator of the Wnt pathway, namely GSK3ß. Additionally, GSK3ß has been recently proposed to be the connecting link between the two pathological AD markers, namely the tau protein containing neurofibrillary tangles (NFT) and the ß-amyloid plaques (Llorens-Martín et al., 2014). Elevated GSK3β activity hasa direct link to increased Aβ production and Aβ deposits, tau hyperphosphorylation, and synaptic damage in AD patients (Reddy et al., 2000; Benedetti et al., 2013). Although evidence on the direct interactions of sFRPs on regulating Wnt activity in the AD brain is lacking, their functional role in attenuating ß-catenin signaling has been demonstrated to be via GSK3ß (Bovolento et al., 2010). Moreover, sFRPs are reported to be involved in the

decline of both cytoplasmic and membrane associated ß-catenin in other cellular contexts that include pancreatic islets (Lee et al., 2008), retinal cells (Esteve et al., 2003), and caudal neural tubes (Dann et al., 2001). Conversely, an sFRP1 deletion study in mice demonstrated reinstated Wnt pathway activation and signaling (Gong et al., 2014), suggesting the potential involvement of these proteins in Wnt related functions. Unlike Dkks, sFRPs can interact with both ß-catenin dependent and independent Wnt signaling pathways (Marchetti and Pluchino, 2013; Muley et al., 2010). Therefore, in an sFRP over-expressed system, they antagonize Wnt actions either by binding directly to Wnts or competing with FZD receptors, and thus preventing Wnt–FZD interactions. As a result of these interactions, the GSK3ß enzyme in the destruction complex targets ß-catenin to ubiquitin-mediated degradation cycles. One can speculate that this inhibition of Wnt/ß-catenin-mediated signaling could result in the accumulation of tau protein and the formation of NFT in the AD brain. This, in turn, disrupts nuclear localization of ß-catenin and activation of the TCF/LEF promoter, which initiates apoptosis that could lead to neuronal instability and formation of amyloid plaques (Fig.2). Subsequent depletion of ß-catenin levels has been correlated to the presence of amyloid plaques during the progressive phases of AD (Maguschak and Ressler, 2012). Thus, the previously observed inverse relation between ß-catenin signaling and GSK3ß activity in AD brains (Avrahami et al., 2013; Hernández et al., 2010) and rat hippocampal neurons (Salcedo-Tello et al., 2011) leads us to speculate that there is another possible involvement of sFRPs as mediators in deregulating the Wnt signaling pathway. Another study demonstrated that Wnt-5a was able to reduce synaptic damage and protect against memory loss in AD, and that this beneficial effect was blocked by sFRP2 (Zhang et al., 2015). Although further substantiation of their functions in neuro-regulatory activities is required, one can expect to recapitulate sFRP-mediated events in the AD brain similar to the reported functions of Dkk-1 (Llorens-Martín et al., 2014), but with wider implications because of the involvement of multiple

cascades of the Wnt pathway in sFRP signal transduction. Hence, it would be worthwhile to consider sFRPs in a wider aspect to understand their mechanistic role as Wnt inhibitors in neuroderegulation.

sFRPs as potential therapeutic targets for AD treatment

In comparison to Dkk-1 or other Wnt inhibitors, sFRP-based targeted strategies are in the early stages of investigation (Fontenot et al., 2013). Expression profiling studies of sFRPs have demonstrated their up-regulation in most neurological diseases (Esteve et al., 2011; Stuckenholz et al., 2013). However, no study has explicitly identified the neuro-regulatory activities of sFRPs in Alzheimer's progression. Given the complexity of the action of sFRPs on Wnt signaling, it is possible this family of Wnt mediators could have a diverse effect on AD progression. Depending on their expression levels and cellular context within the CNS, sFRPs could simultaneously interact with Wnt and FZD to elicit varied responses. A systematic delineation of expression the sFRP family of proteins in AD progression would help to determine sFRPs as stage-specific biomarkers and as therapeutic targets in the degenerative neuropathology of AD. As sFRPs have a broad effect across the multiple pathways of Wnt signaling, such an approach would help in a wider capture of the neurodegeneration caused by a dysfunctional Wnt machinery. A deeper understanding of the molecular mechanism of action of sFRPs in Wnt regulation, in the context of Alzheimer's neurodegeneration, could provide a framework for designing novel sFRP-targeted therapies in AD.

The effect of sFRP on Wnt signaling in the CNS is most likely to be inhibitory because of its binding to both the ligand and the receptor. The binding to FZD by the CRD domain of sFRP may not be sufficient to elicit the complex assembly of the Axin conglomerate, required for the downstream activation of ß -catenin. The binding at the FZD receptor could be further

exacerbated by Aß also binding to FZD close to the binding site of Wnt. Targeting the sFRPs to relieve its suppression on the Wnt pathway could be achieved by designing small molecule inhibitors for different sFRPs, similar to the diarylsulfone sulphonamide inhibitor for sFRP1 (Bodine et al., 2009), obtained by high throughput screening.

Conclusion

The need to identify dysfunctional regulatory networks in AD is compelling in order to design novel and effective drug targets. As the present modalities in AD therapies are mostly aimed at administering symptomatic treatments, there is a direct need for causative targeting of the disease, which will not only help in AD regression but also in early pre-symptomatic identification of the disease. With the advent of early biomarkers for AD related to the Wnt pathway, there could be hope for more accurate and early diagnosis and promising therapy for this debilitating disease. Therefore, we propose that sFRPs could have a profound role in AD and could be exploited as novel biomarkers for early detection. Targeting sFRPs might be a more promising strategy than other inhibitors such as GSK3ß or DKK because of the ability of sFRPs to modulate the multiple Wnt-driven signaling events. Furthermore, as a corollary, targeting their expression could bring about amelioration of neurodegenerative changes associated with AD.

Conflict of Interest

The authors declare no conflict of interest.

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FIGURE LEGENDS

Fig 1. The β-catenin dependent and independent pathways of Wnt signaling

Extracellular ligand Wnt acts through the β-catenin dependent or via the β-catenin independent pathways, through the Frizzled (FZD) receptor and via the activation of intracellular disheveled (DVL) or heteromeric G-proteins. In the β-catenin dependent pathway, β-catenin is activated and transported to the nucleus for downstream activation

of Wnt target genes via the T-cell factor/Lymphoid enhancer factor (TCF/LEF) promotor. In the β-catenin independent pathway, binding of Wnt to FZD activates either the Ca^{2+} pathway or the planar cell polarity pathway, which are involved in cell survival and cytoskeletal formation through actin regulation. (CAMK-Calcium/calmodulin-dependent protein kinase, protein kinase C - PKC, Jun-N-terminal kinase- JNK, Activating transcription factor 2- ATF-2, cAMP-response element-binding protein-CREB, N-Methyl-D-aspartate receptors- NMDAR, post synaptic density-95- PSD-95).

Fig 2. A model for sFRP-mediated inhibition of the Wnt signaling pathways in Alzheimer's disease

In the Alzheimer's brain, over-expressed sFRP could bind to Wnt and Frizzled (FZD) to destabilize the axin complex and release GSK3ß from the complex, which in turn phosphorylates ß-catenin and Tau protein, increases Aß production, and stalls TCF/LEF-initiated transduction of pro-proliferative genes. This could result in the accumulation of Tau and ß-amyloid proteins, and the loss of synaptic integrity and neuronal damage. Dysregulation of the Wnt/Ca^{2+} pathway could cause disruption in the N-Methyl-D-aspartate receptors and accumulation of extracellular glutamate.