PNAS COMMENTARY

An intrinsically disordered protein, osteopontin, driving neuropathology in Alzheimer's dementia

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Osteopontin (OPN), a protein lacking a fixed structure and thus considered "intrinsically disordered," (1) may play a key role in Alzheimer's disease (AD), characterized by both disordered memory and profound loss of cognitive function. In this issue of the Proceedings, Qiu and Shen along with their colleagues show that in AD, levels of OPN produced in microglia bearing the $CD11c^+$ cell surface marker are correlated with the severity of cognitive deficits (2). In addition, OPN levels in the AD brain reflect the intensity of neuropathologic damage. Higher levels of OPN in the brain (2) and CSF (3) and increased $CD11c^+$ OPN^+ microglia in the brain (2) are associated with a higher density of neurofibrillary tangles and neuritic plaques, two neuropathologic hallmarks of this tragic disease. Working in an animal model of AD, the investigators identified two types of microglia that were $CD11c^+$. The two categories of microglia have opposing properties: one type, producing high amounts of OPN were pathogenic with worsening Alzheimer's like features in the animal model, while $CD11c^+$ microglia producing low OPN were protective (2).

OPN is a member of a family of glycophosphoproteins (4). The genes and the proteins they encode within this family play important roles in bone physiology including prominent roles in dental health (5). The proteins grouped near one another on chromosome 4-OPN, bone sialoprotein, dentin matrix protein 1, dentin sialophosphoprotein, and matrix extracellular phosphoglycoprotein are members of the small integrin-binding ligand N-linked glycoproteins (SIBLINGs) family. All members have RGD motifs, similar to fibronectin, vitronectin, and fibrinogen, and thus bind to various integrins (4, 6). Two winners of the Lasker Prize in 2022 for their work on integrins played significant roles in this story. Richard Hynes was the first to identify this protein, first called transformation-specific phosphoprotein in a study on induced transformed cells in 1979 (7). Erkki Ruoslahti first described the RGD motif necessary for binding integrins in 1984 (8).

OPN is a protein with a name that might make one think that it was related to the bones in the skull that encase and protect the brain, and not to the most common degenerative disease of the brain itself. In fact, OPN has key roles in other neurologic diseases including multiple sclerosis (6, 9, 10), amyotrophic lateral sclerosis (10, 11), and stroke (10). Oldberg et al. were the first to clone, sequence, and then name the protein OPN in 1986. They wrote that "we suggest that the protein is named OPN, denoting that it is a product of cells in the osteoid matrix and that it can form a bridge (in Latin, pons) between cells and the mineral in the matrix" (12). OPN is officially named secreted phosphoprotein 1 (SPP1). Nevertheless, Harvey Cantor working on this protein and its role in the immune system referred to OPN in a 1987 paper as ETA-1, "early T cell activation -1" protein (13). Maybe we should name proteins with mere letters and numbers and endeavor to avoid colorful names. After all, one of the most boring names for a protein "Tissue Factor" is immensely important in the coagulation cascade. It would be more intriguing perhaps if Tissue Factor were just named CD142. Who wants to tell their friends, unless they are hematologists, that they study "tissue factor?" Mentioning CD142 would more likely elicit curiosity, rather than mentioning its given name. Of course, all proteins are important and have remarkable stories, regardless of their name! If one is to name a protein, at least provide a name that sparkles. OPN is an intriguing and shiny name, and yet, it might first raise doubt about how it could play a role in AD, a disease of the brain, not bone.

The investigators described in great detail the role for OPN in AD. They studied human brain specimens from individuals with AD and then provided mechanistic insights from experiments they performed using the elegant transgenic mouse model known as 5XFAD (14). This transgenic mouse has overexpression of two human proteins APP and PS1, sometimes associated with inherited forms of AD. The 5XFAD mouse has transgenes for five of these mutant proteins from familial AD mutations (14).

The investigators first isolated microglia from 5XFAD brains, using a stringent protocol to purify microglia. One of the daunting challenges in the field is to separate the blood-borne macrophages from the microglia in the brain (15). In any case, the investigators focused on two $CD11c^+$ subsets that they identified, that had differential expression of OPN. They also created a "loss-of-function" mutation in a mouse effectively knocking out OPN in the 5XFAD mice, named OPN-KO.5XFAD. These mice had fewer abnormal neurites. The abnormal neurites had defective bulbous axons and pieces of synapses containing amyloid precursor protein, all signs of AD. The OPN-KO.5XFAD mice performed better than 5XFAD mice on different forms of behavioral testing.

Of interest, the microglia producing OPN, also produce large amounts of TNF. A study of microglia populations with CyToF technology revealed that tumor necrosis factor-a (TNF-α) was the most prominent of eight cytokines studied in microglia. This was seen in models of neuroinflammation

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and in models of Huntington's disease and amyotrophic lateral sclerosis. An AD model was not tested in that study (15). In the 5XFAD mice, where OPN was knocked out, there was a 50% reduction in TNF-α.

Amyloid-beta (Aβ) is considered one of the pathogenic drivers of AD. In fact, removal of toxic Aβ has been the dominant hypothesis in the AD field, and major clinical trials have shown glimmers of promise in a few well-publicized instances. The $CD11c^+$ OPN^+ microglia phagocytose less Aβ. The $CD11c^+OPN-$ microglia do not produce significant levels of TNF-α and engulf greater amounts of Aβ. These protective microglia express markers that indicate that they have increased lysosomal activation and thus ingest more of the toxic Aβ presumably for disposal, before neurons are damaged. It will be interesting to test whether these $CD11c^+OPN-$ microglia preferentially ingest soluble oligomers and protofibrils of Aβ, that may be driving the pathology in AD (16).

"Qiu et al. show that in AD, levels of OPN produced in microglia bearing the CD11c+ cell surface marker are correlated with the severity of cognitive deficits."

It is noteworthy that the dominant hypothesis in AD—that Aβ toxicity is the root of neuropathology—does have a Janus face. There is abundant evidence from both gain-of-function and loss-of-function experiments that there are "protective amyloid" structures that mitigate neuroinflammation and neurodegeneration. Investigators have identified amyloid-forming hexapeptide units in many proteins including Aβ, that activate a nicotinic AChR. The α7 nAChR activates a well-known pathway that suppresses inflammatory responses. Activation of this pathway is highly protective in neuroinflammatory conditions (17, 18). The reconciliation of the impact of these opposing effects of amyloidogenic structures on the nervous system, providing both protection in some circumstances and triggering destruction in other instances, will continue to be a fascinating and controversial subject in diseases involving neuroinflammation and neurodegeneration (2, 18, 19).

Qiu and Shen and their colleagues have opened a major new opportunity for potential breakthrough therapies for AD. Such therapies may augment antiamyloid approaches as we learn more about the exact molecular species of Aβ oligomers that are pathogenic versus conformations of Aβ which confer protection. The amyloid hypothesis has dominated the field for a generation, and yet, even its most ardent proponents would likely admit that adjunctive therapy will be needed to address aspects of neuroinflammation that accompany neurodegeneration. By administration of an antibody to OPN, the investigators were able to reduce the extent of Aβ plaque pathology characteristic of AD. The results with administration of the monoclonal antibody OPN weekly and via an intravenous route were impressive, but more effective dosing and augmentation of delivery of the monoclonal antibody to the brain may further improve outcomes.

Small-molecule inhibitors with better blood–brain barrier penetration may give even more striking results than the monoclonal antibody targeting OPN, given that only a few percent of monoclonal antibody given intravenously can penetrate the blood–brain barrier in AD and in its animal models. The fact that amelioration of pathology was observed with a monoclonal to an intrinsically disordered protein, such as OPN, is encouraging. Perhaps locking in a pathologic conformation of OPN, if one can be discerned, is worthy of further consideration for designing optimized biologics and small molecules that address this fascinating therapeutic target. It is ironic that a disordered protein may be a critical molecule in modulating a disease that creates massive dysfunction and disorder in the brains of individuals and leads to dementia, known as AD (2).

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