α 2-macroglobulin in Alzheimer's disease: new roles for an old chaperone

Sahba Seddighi¹, Vijay Varma¹ & Madhav Thambisetty^{*,1}

¹Clinical & Translational Neuroscience Unit, Laboratory of Behavioral Neuroscience, National Institute on Aging (NIA), National Institutes of Health (NIH), Baltimore, MD 21224, USA

* Author for correspondence: Tel.: +1 410 558 8572; Fax: +1 410 558 8674; thambisetty@mail.nih.gov

“our findings demonstrate a network of tau-phosphorylation-sensitive genes that are associated with expression of A2M and, furthermore, implicate systemic inflammation as a key player in RCAN1–calcineurin–NFAT signaling”

First draft submitted: 22 January 2018; Accepted for publication: 6 February 2018; Published online: 14 March 2018

Keywords: α 2-macroglobulin • Alzheimer's disease • biomarkers • cerebrospinal fluid • chaperone • inflammation • neuroimaging • plasma

Extracellular chaperone proteins are essential components of proteostasis control through their ability to facilitate proper protein folding as well as the sequestration and disposal of terminally misfolded species [1]. The aggregation of misfolded amyloid- β (A β) peptides is a central hallmark of Alzheimer's disease (AD) that is believed to trigger a cytotoxic cascade of events leading to synaptic dysfunction, neuronal death and cognitive impairment [2,3]. Accordingly, chaperone proteins, which assist in the disassembly and clearance of proteins, have received considerable attention as biomarkers and potential therapeutic targets for the treatment of AD [4–6]. As detailed in our previous review, prior studies have largely focused on the interaction of chaperone proteins with neurotoxic A β , while their interactions with other pathogenic proteins in AD, such as tau, remain less well understood [7]. With the recent failure of multiple Phase III anti-A β immunotherapy clinical trials [8–10], a review of recent advances in understanding the interactions of chaperone proteins with other pathological features of AD is timely.

α 2-macroglobulin as a marker of neuronal injury in AD

In addition to helping proteins to achieve their native states, chaperones can regulate cytokine secretion as well as leukocyte migration and are thereby involved in a multitude of inflammatory functions [11]. Although some chaperones, such as Hsp27 and members of the Trx superfamily, exert anti-inflammatory effects, others including cyclophilin are proinflammatory in nature, while still others, such as Hsp60 and Hsp70 proteins, can exert both effects [11].

A growing body of literature supports the significant contribution of neuroinflammation to the pathogenesis of AD [12,13]. However, the role of systemic inflammation in the onset and progression of AD remains unclear. To better characterize this relationship, we recently examined whether α 2-macroglobulin (A2M), a well-characterized chaperone protein and an acute phase reactant of the innate immune system [14,15], may be involved in the pathogenesis of preclinical AD [16]. We showed that plasma A2M concentration is significantly associated with cerebrospinal fluid markers of neuronal injury, in other words, total-tau and phosphorylated tau. Moreover, we found that higher baseline serum concentration of A2M in cognitively normal individuals is associated with a greater risk of progression to clinical AD in men – suggesting a sex-specific variation in the inflammatory response during the early stages of AD pathogenesis.

A2M as a marker of tau phosphorylation in AD

In the same study, we utilized a system-level approach combining gene expression and proteomic datasets to identify a network of nine coregulated genes whose expression jointly predicts that of the *A2M* gene. Interestingly, this gene network includes regulator of calcineurin (*RCAN1*) whose target effector, calcineurin, is a well-characterized tau

phosphatase. Moreover, calcineurin is a calcium/calmodulin-dependent phosphatase that functions to dephosphorylate the transcription factor, nuclear factor of activated T cells (NFAT). Upon activation, NFAT translocates to the nucleus, triggering a cascade of transcriptional events that lead to the production of endogenous cytokines [17]. Previous studies have demonstrated that calcineurin–NFAT signaling is involved in the pathogenesis of neurodegenerative diseases. However, the precise relationship between RCANs and calcineurin–NFAT activity appears to be variable and context-dependent [18,19], with specific isoforms of RCAN capable of both inhibiting and facilitating calcineurin–NFAT signaling [20]. Linking A2M with RCAN1–calcineurin signaling, our recent results show that gene expression of *A2M* is positively correlated with RCAN1 and negatively correlated with calcineurin in the brain [16]. Together, our findings demonstrate a network of tau-phosphorylation-sensitive genes that are associated with expression of A2M and, furthermore, implicate systemic inflammation as a key player in RCAN1–calcineurin–NFAT signaling. Though the precise nature of these interactions awaits further study, we speculate that A2M, as a chaperone of aggregation-prone proteins, is upregulated in response to tau phosphorylation secondary to the downregulation of calcineurin activity. Although chaperone proteins are best recognized for their extracellular functions, the interaction of A2M with tau may occur intracellularly. In neurons, the disruption of metabolic homeostasis and the buildup of misfolded proteins – both key features of AD pathogenesis – lead to endoplasmic reticulum stress, which can trigger the unfolded protein response [21–23]. Though the unfolded protein response pathway is initially protective, it can become constitutively active and neurotoxic in a number of neurodegenerative tauopathies, including AD. Interestingly, the activation of this pathway is accompanied by an increase in chaperone activity and, subsequently, tau phosphorylation, providing a plausible mechanism through which A2M may interact with tau [24–27].

A2M & synaptic failure in AD

In addition to *RCAN1*, another interesting component of the *A2M* gene network that we reported [16] is secreted protein, acidic and rich in cysteine-like1 (*SPARCL1*, also known as hevin). *SPARCL1* encodes an astrocyte-secreted synaptogenic protein that is markedly upregulated following injury in the nervous system to aid in neural circuit remodeling [28–30]. Moreover, *SPARCL1*/hevin has recently been shown to play a key role in glutamatergic synaptogenesis during development [31]. We have recently shown that polymorphic variation in the *SPARCL1* gene is associated with several AD endophenotypes, including accelerated cognitive decline, faster brain atrophy and altered neuronal activity in several brain regions [32]. Taken together, our findings suggest that *SPARCL1* and A2M may respond to neuronal injury in preclinical AD. A detailed understanding of these potential interactions may further clarify whether modulating A2M activity may be a promising experimental therapeutic approach in AD.

A2M as a therapeutic target in AD & autoimmune diseases

Wyatt and colleagues have shown that A2M inhibits amyloid formation through its bait-and-trap chaperone activity [33] and that hypochlorite, an oxidant produced *in vivo* by the innate immune system, can dramatically enhance the A β clearing abilities of A2M [34]. In addition, Yerbury and Wilson have demonstrated that the introduction of exogenous A2M can protect cultured neuroblastoma cells from A β -induced neurotoxicity [35].

Recent studies suggest that A2M may also be a promising target for the treatment of other degenerative and inflammatory conditions. Wang and colleagues have established that A2M levels are lower in synovial fluid from patients with osteoarthritis, and that supplemental injection of A2M attenuates post-traumatic progression of osteoarthritis in rats [36]. In line with these findings, a recent study has shown that A2M can inhibit inflammatory mediators in rheumatoid arthritis, thereby exerting a chondroprotective effect [37]. A detailed understanding of the immunomodulatory properties of A2M in such conditions merits further study, as it may help guide future efforts to develop disease-altering therapies for the treatment of a wide range of degenerative and autoimmune disorders.

To this end, several key questions remain to be addressed: first, what is the precise role of systemic inflammation and A2M in the regulation of RCAN1–calcineurin–NFAT signaling, and to what degree may such regulation be beneficial or harmful? Second, how might A2M influence calcineurin/NFAT to respond to or affect tau phosphorylation? And finally, can the modulation of A2M serve as an effective therapeutic strategy in neurodegenerative diseases, such as AD?

Financial & competing interests disclosure

This work was supported by funding from the intramural research program of the National Institute on Aging, NIH. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

- Hartley SL, Barker ET, Seltzer MM *et al*. The relative risk and timing of divorce in families of children with an autism spectrum disorder. *J. Fam. Psychol.* 24(4), 449–457 (2010).
- Nimmrich V, Ebert U. Is Alzheimer's disease a result of presynaptic failure? Synaptic dysfunctions induced by oligomeric beta-amyloid. *Rev. Neurosci.* 20(1), 1–12 (2009).
- Lublin AL, Gandy S. Amyloid-beta oligomers: possible roles as key neurotoxins in Alzheimer's disease. *Mt Sinai J. Med.* 77(1), 43–49 (2010).
- Thambisetty M, Simmons A, Velayudhan L *et al*. Association of plasma clusterin concentration with severity, pathology and progression in Alzheimer disease. *Arch. Gen. Psychiatry* 67(7), 739–748 (2010).
- Wilson MR, Yerbury JJ, Poon S. Potential roles of abundant extracellular chaperones in the control of amyloid formation and toxicity. *Mol. Biosyst.* 4(1), 42–52 (2008).
- Kolstoe SE, Ridha BH, Bellotti V *et al*. Molecular dissection of Alzheimer's disease neuropathology by depletion of serum amyloid P component. *Proc. Natl Acad. Sci. USA* 106(18), 7619–7623 (2009).
- Thambisetty M. Do extracellular chaperone proteins in plasma have potential as Alzheimer's disease biomarkers? *Biomark. Med.* 4(6), 831–834 (2010).
- Iqbal K, Liu F, Gong CX. Alzheimer disease therapeutics: focus on the disease and not just plaques and tangles. *Biochem. Pharmacol.* 88(4), 631–639 (2014).
- Ising C, Stanley M, Holtzman DM. Current thinking on the mechanistic basis of Alzheimer's and implications for drug development. *Clin. Pharmacol. Ther.* 98(5), 469–471 (2015).
- Mehta D, Jackson R, Paul G, Shi J, Sabbagh M. Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010–2015. *Expert Opin. Invest. Drugs* 26(6), 735–739 (2017).
- Henderson B, Pockley AG. Molecular chaperones and protein-folding catalysts as intercellular signaling regulators in immunity and inflammation. *J. Leukoc. Biol.* 88(3), 445–462 (2010).
- Eikelenboom P, Veerhuis R, Van Exel E, Hoozemans JJ, Rozemuller AJ, Van Gool WA. The early involvement of the innate immunity in the pathogenesis of late-onset Alzheimer's disease: neuropathological, epidemiological and genetic evidence. *Curr. Alzheimer Res.* 8(2), 142–150 (2011).
- Heneka MT, Carson MJ, El Khoury J *et al*. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 14(4), 388–405 (2015).
- Armstrong PB, Quigley JP. Alpha2-macroglobulin: an evolutionarily conserved arm of the innate immune system. *Dev. Comp. Immunol.* 23(4–5), 375–390 (1999).
- French K, Yerbury JJ, Wilson MR. Protease activation of alpha2-macroglobulin modulates a chaperone-like action with broad specificity. *Biochemistry* 47(4), 1176–1185 (2008).
- Varma VR, Varma S, An Y *et al*. Alpha-2 macroglobulin in Alzheimer's disease: a marker of neuronal injury through the RCAN1 pathway. *Mol. Psychiatry* 22(1), 13–23 (2017).
- Bell J. T-cell turn-off. *Nat. Rev. Immunol.* 2(7), 460–460 (2002).
- Mojas B, Mora S, Bossowski JP, Lassot I, Desagher S. Control of neuronal apoptosis by reciprocal regulation of NFATc3 and Trim17. *Cell Death Differ.* 22(2), 274–286 (2015).
- Boczek T, Ferenc B, Lisek M, Zylinska L. Regulation of GAP43/calmodulin complex formation via calcineurin-dependent mechanism in differentiated PC12 cells with altered PMCA isoforms composition. *Mol. Cell. Biochem.* 407(1–2), 251–262 (2015).
- Kipanyula MJ, Kimaro WH, Seke Etet PF. The emerging roles of the calcineurin-nuclear factor of activated T-lymphocytes pathway in nervous system functions and diseases. *J. Aging Res.* 2016, 5081021 (2016).
- Frisardi V, Solfrizzi V, Seripa D *et al*. Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease. *Ageing Res. Rev.* 9(4), 399–417 (2010).
- Chiti F, Dobson CM. Protein misfolding: amyloid formation and human disease: a summary of progress over the last decade. *Annu. Rev. Biochem.* 86, 27–68 (2017).
- Walter P, Ron D. The unfolded protein response from stress pathway to homeostatic regulation. *Science* 334(6059), 1081–1086 (2011).
- Nijholt DA, Van Haastert ES, Rozemuller AJ, Scheper W, Hoozemans JJ. The unfolded protein response is associated with early tau pathology in the hippocampus of tauopathies. *J. Pathol.* 226(5), 693–702 (2012).

- 25 Hoozemans JJ, Veerhuis R, Van Haastert ES *et al.* The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol.* 110(2), 165–172 (2005).
- 26 Schroder M, Kaufman RJ. ER stress and the unfolded protein response. *Mutat. Res.* 569(1–2), 29–63 (2005).
- 27 Van Der Harg JM, Nolle A, Zwart R *et al.* The unfolded protein response mediates reversible tau phosphorylation induced by metabolic stress. *Cell Death Dis.* 5, e1393 (2014).
- 28 Kucukdereli H, Allen NJ, Lee AT *et al.* Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC. *Proc. Natl Acad. Sci. USA* 108(32), E440–E449 (2011).
- 29 Eroglu C. The role of astrocyte-secreted matricellular proteins in central nervous system development and function. *J. Cell Commun. Signal.* 3(3–4), 167–176 (2009).
- 30 Jones EV, Bouvier DS. Astrocyte-secreted matricellular proteins in CNS remodelling during development and disease. *Neural Plast.* 2014, 321209 (2014).
- 31 Singh SK, Stogsdill JA, Pulimood NS *et al.* Astrocytes assemble thalamocortical synapses by bridging NRX1alpha and NL1 via Hevin. *Cell* 164(1–2), 183–196 (2016).
- 32 Seddighi SVV, An Y, Varma S *et al.* SPARCL1 accelerates symptom onset in Alzheimer's disease and influences brain structure and function during aging. *J. Alzheimer's Dis.* 61(1), 401–414 (2017).
- 33 Wyatt AR, Constantinescu P, Ecroyd H *et al.* Protease-activated alpha-2-macroglobulin can inhibit amyloid formation via two distinct mechanisms. *FEBS Lett.* 587(5), 398–403 (2013).
- 34 Wyatt AR, Kumita JR, Mifsud RW, Gooden CA, Wilson MR, Dobson CM. Hypochlorite-induced structural modifications enhance the chaperone activity of human alpha2-macroglobulin. *Proc. Natl Acad. Sci. USA* 111(20), E2081–E2090 (2014).
- 35 Yerbury JJ, Wilson MR. Extracellular chaperones modulate the effects of Alzheimer's patient cerebrospinal fluid on Aβ1–42 toxicity and uptake. *Cell Stress Chaperones* 15(1), 115–121 (2010).
- 36 Wang S, Wei X, Zhou J *et al.* Identification of alpha2-macroglobulin as a master inhibitor of cartilage-degrading factors that attenuates the progression of posttraumatic osteoarthritis. *Arthritis Rheumatol.* 66(7), 1843–1853 (2014).
- 37 Cuellar JM, Cuellar VG, Scuderi GJ. Alpha2-macroglobulin: autologous protease inhibition technology. *Phys. Med. Rehabil. Clin. N. Am.* 27(4), 909–918 (2016).