Expression of the TGF-β1/p53 Target SERPINE1 Gene in Alzheimer's Dementia: Molecular Mechanisms and Therapeutic Opportunities

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INTRODUCTION

Cardiovascular, thrombotic and neurodegenerative diseases significantly increase with age. These disorders are likely the result of ageing-related pathophysiologic changes in the vascular, hemostatic and central nervous systems (CNS) reflecting the development of coagulation anomalies, advanced sclerotic changes and deficiencies in protein degradation and clearance. Accumulation of neuronal tangles and amyloid peptides (Aβ) is a major cause of age-onset dementia and a hallmark neuropathologic feature of Alzheimer's disease (AD) for which there is currently no effective treatment. The plasmin-generating cascade, involving urokinase (uPA) and tissue-type (tPA) plasminogen activators, convert plasminogen to the broad-spectrum protease plasmin. Plasmin provides an Aβ-clearing function in the brain degrading Aβ and catalyzing amyloid precursor protein (APP) α- site APP proteolysis producing nontoxic peptides. Plasmin activation, in turn, is negatively regulated by the clade E, member 1, serine protease inhibitor PAI-1 (plasminogen activator inhibitor type-1; SERPINE1) resulting in Aβ accumulation. PAI-1 and its major physiological inducer transforming growth factor-β1 (TGF-β1), moreover, are both increased in animal models of Alzheimer's disease as well as implicated in the development of human neurodegenerative processes. Importantly, direct injection of the pathogenic $A\beta_{40}$ peptide into the CA1 region of the hippocampus significantly up-regulated PAI-1 expression in $tPA^{-/-}$ and plasminogen-deficient mice but only weakly in wild-type animals. This suggests the existence of a mechanism that, once initiated, exacerbates disease progression. Targeting of PAI-1 function and/or expression may constitute a clinically-relevant molecular approach to the therapy of age-related neurodegenerative diseases associated with increased PAI-1 levels.

THE PLASMINOGEN SYSTEM IN ALZHEIMER'S DISEASE

Aggregated β-amyloid peptides accumulate in plaque-like structures in specific areas of the brain in AD patients by proteolytic processing of the single-pass transmembrane APP [1]. These deposits initiate prolonged CNS inflammation, neuronal death, and eventual cognitive decline [2]. Aβ peptides are produced by aspartic protease (BACE)-induced β-site cleavage of APP and subsequent proteolysis (*via* presenilin and nicastrin) at the C99 transmembrane-localized γ position [3–6]. The broad-spectrum protease plasmin degrades Aβ [7–9] and plasminogen →plasmin activation decreases Aβ peptide levels [10]. While the mechanisms are complex, plasmin-mediated proteolysis of APP at the α-site (either as a direct or indirect target; the latter as a consequence of plasmin activation of matrix metalloproteinases including TACE or ADAM 10) generating the non-toxic p3 peptide [3,6] resulting in decreased Aβ production. Collectively, these data suggest a protective role for the plasmin cascade in the CNS. Plasmin levels in the brains of AD patients are, in fact, considerably reduced [10] supporting a causal relationship between deficient activity of the plasmin-generating proteolytic system and accumulation of Aβ in the progression of AD.

GENETIC APPROACHES IMPLICATE PAI-1 IN AMYLOID ACCUMULATION

Several members of the SERPIN superfamily (SERPINF1, SERPINI1, SERPINE1 (PAI-1), SERPINE2, and SERPINA3) exhibit cell-type neuro-trophic, neuroprotective, or neuropathophysiologic activities [11]. PAI-1 (SERPINE1), in particular,

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appears to have multifunctional roles in the CNS where it maintains neuronal cellular structure and initiates signaling through mitogen-activated protein kinases [12]. Recent findings, moreover, suggest a more global impact on intracellular networks as PAI-1 also activates the Jak/Stat and Akt pathways by binding to the low-density lipoprotein receptor-related protein-1 (LRP-1), a member of the low density lipoprotein (LDL) receptor gene family [Czekay, Archambeault and Higgins, unpublished data]. Whether these varied effects are dependent on the anti-proteolytic function of PAI-1 is not clear but significantly increased PAI-1 immunoreactivity in the CNS of AD patients is associated with development of senile plaques and ghost tangle structures [13], consistent with the colocalization of plasminogen activators and PAI-1 in plaque structures [14] which are sites of sustained inflammation [15]. Tg2576 and TgCRN8 transgenic mice, that are genetically-engineered to express the brain-targeted Swedish and the double Swedish/V717F Aβ mutants, respectively, exhibit age-dependent Aβ plaque development as well as cognitive deficiencies [16]. Importantly, tPA activity in these mice was specifically decreased significantly in the hippocampus and amygdala which correlated corelating with regional increases in PAI-1 expression [17]. Since direct Aβ peptide injection increased PAI-1 expression and whereas Aβ hippocampal clearance required both tPA and plasminogen, a functional tPA-plasmin axis appears necessary for Aβ removal [17]. While PAI-1 may be neuroprotective in specific settings (e.g., tPA-triggered neuronal apoptosis) [18,19] and is a CNS injury-response gene [20], chronically elevated PAI-1 levels nevertheless promote Aβ accumulation by inhibiting plasmin-dependent degradation. Genetic evidence clearly indicates that brain PAI-1 expression is increased in Aβ precursor protein presenilin 1 (APP/PS1) transgenic mice as well as in AD patients [21] while PAI-1-deficiency in an APP/PS1 background reduces amyloid accumulation likely by increasing tPA and plasmin activities [22]. Indeed, a diet containing the phenolic anti-oxidant tert-butylhydroquione reduced brain Aβ load in APP/PS1 transgenics and inhibited PAI-1 expression [22]. The translational impact of this study is highlighted by the realization that TGF-β1-induced PAI-1 gene expression is dependent on the generation of reactive oxygen species by p22(Phox)-containing NADP(H) oxidases [23,24].

THERAPEUTIC OPPORTUNITIES

The development of pharmacologic approaches to inhibit the function of a key contributor (PAI-1) to disease progression has significant translational relevance. AD patients have elevated neuronal levels of tPA, uPA, PAI-1 and α2-antiplasmin where they associate with Aβ plaques; their offsetting activities may blunt plasmin generation and inhibit Aβ clearance [25]. Importantly, a small molecule inhibitor of PAI-1 activity (PAZ-417) partially blocks amyloid deposition in a mouse AD model. PAI-1 inhibition stimulates tPA/plasmin activity, decreasing CNS Aβ levels and reverses cognitive deficits [26] suggesting that such targeting may have clinical utility. In addition, histone deacetylase inhibitors (HDACi) are emerging as a promising therapy for neurodegenerative disease [27]. Sodium butyrate (NaB), a broad-spectrum HDACi, improved learning and memory in rats subjected to the standard Morris water maze challenge [28]. Butyrate localizes to the cerebral cortex in KCl-induced spreading cortical depression [29]. NaB (as well as TSA) are neuroprotective in the context of ischemic brain injury [30] and effectively reduced TGF-β1-induced PAI-1 expression [19]. Brain TGF-*β*1 levels increase during the onset and progression of Parkinson's disease, AD, and stroke [reviewed in 19]. Elevated TGF-*β*1 expression correlates with A*β* angiopathy and transgenic mice that overexpress TGF-*β* in astrocytes exhibit early onset A*β* deposition [31]. TGF-*β*1, moreover, induces astrocyte APP expression while A*β* production was enhanced by TGF-*β*1 signaling [32]. The coordinate overexpression of PAI-1 and increased A*β* generation in response to elevated TGF-*β*1 in AD patients may dispose to disease progression [33]. Collectively, these findings raise the possibility that targeting specific TGF-*β*1-inducible genes (e.g., PAI-1, APP) may have therapeutic benefit in the setting of AD. HDACi coupled with a small molecule central nervous system-accessible PAI-1 functional inhibitor may have efficacy as an approach to reverse the ongoing accumulation of amyloid deposits even after disease development.

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REFERENCES

[1] Glenner GG, Wong CW. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun 1984; 122: 1131-1135. http://dx.doi.org/10.1016/0006-291X(84)91209-9

[2] Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature 1999; 399(6738 suppl): A23-A31. http://dx.doi.org/10.1038/399a023

[3] Parvathy S, Hussain I, Karran EH, Turner AJ, Hooper NM. Cleavage of Alzheimer's amyloid precursor protein by α-secretase occurs at the surface of neuronal cells. Biochemistry 1999; 38: 9728-9734. http://dx.doi.org/10.1021/bi9906827

[4] Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, *et al*. β-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 1999; 286: 735-741. http://dx.doi.org/10.1126/science.286.5440.735

[5] Yu G, Nishimura M, Arawaka S, Levitan D, Zhang L, *et al*. Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and βAPP processing. Nature 2000; 407: 48-54. http://dx.doi.org/10.1038/35024009

[6] Periz G, Fortini ME. Proteolysis in Alzheimer's disease: can plasmin tip the balance? EMBO Reports 2000; 1: 477-478. http://dx.doi.org/10.1093/embo-reports/kvd124

[7] Tucker HM, Kihiko M, Caldwell JN, Wright S, Kawarabayashi T, *et al*. The plasmin system is induced by and degrades amyloid-β aggregates. J Neurosci 2000; 20: 3937-3946.

[8] Van Nostrand WE, Porter M. Plasmin cleavage of the amyloid β-protein: alteration of secondary structure and stimulation of tissue plasminogen activator activity. Biochemistry 1999; 38: 11570-11576. http://dx.doi.org/10.1021/bi990610f

[9] Wnendt S, Wetzels I, Gunzler WA. Amyloid β peptides stimulate tissue-type plasminogen activator but not recombinant prourokinase. Thromb Res 1997; 85: 217-224. http://dx.doi.org/10.1016/S0049-3848(97)00006-6

[10] Ledesma MD, Da Silva JS, Crassaerts K, Delacourte A, De Strooper B, Dotti CG. Brain plasmin enhances APP α-cleavage and Aβ degradation and is reduced in Alzheimer's disease brains. EMBO Reports 2000; 1: 530-535. http://dx.doi.org/10.1093/embo-reports/kvd107

[11] Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, *et al*. The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. J Biol Chem 2001; 276: 33293-33296. http://dx.doi.org/10.1074/jbc.R100016200

[12] Vivien D, Buisson A. Serine protease inhibitors: novel therapeutic targets for stroke? J Cereb Blood Flow Metabol 2000; 20: 755-764. http://dx.doi.org/10.1097/00004647-200005000-00001

[13] Hino H, Akiyama H, Iseki E, Kato M, Kondo H, *et al*. Immunohistochemical localization of plasminogen activator inhibitor-1 in rat and human brain tissues. Neurosci Lett 2001; 297: 105-108. http://dx.doi.org/10.1016/S0304-3940(00)01679-7

[14] Rebeck GW, Harr SD, Strickland DK, Hyman BT. Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein e receptor, the α2-macroglobulin receptor/ low-density-lipoprotein receptor - related protein. Ann Neurol 1995; 37: 211-217. http://dx.doi.org/10.1002/ana.410370212

[15] McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995; 21: 195-218. http://dx.doi.org/10.1016/0165-0173(95)00011-9

[16] Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, *et al*. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science 1996; 274: 99-102. http://dx.doi.org/10.1126/science.274.5284.99

[17] Melchor JP, Pawlak R, Strickland S. The tissue plasminogen activator-plasminogen proteolytic cascade accelerates amyloid-β (Aβ) degradation and inhibits Aβ-induced neurodegeneration. J Neurosci 2003; 23: 8867-8871.

[18] Flavin MP, Zhao G, Ho LT. Microglial tissue plasminogen activator (tPA) triggers neuronal apoptosis *in vitro*. GLIA 2000; 29: 347-354. http://dx.doi.org/10.1002/(SICI)1098-1136(20000215)29:4<347::AID-GLIA5>3.0.CO;2-8

[19] Higgins PJ. The TGF-β1/upstream stimulatory factor-regulated PAI-1 gene: potential involvement and a therapeutic target in Alzheimer's disease. J Biomed Biotechnol 2006: ID15792: 1-6. http://dx.doi.org/10.1155/JBB/2006/15792

[20] Hultman K, Blomstrand F, Nilsson M, Wilhelmsson U, Malmgren K, Pekny M, Kousted T, Jern C, Tjärnlund-Wolf A. Expression of plasminogen activator inhibitor-1 and protease nexin-1 in human astrocytes: Response to injury-related factors. J Neurosci Res 2010; 88: 2441-2449.

[21] Liu RM, van Groen T, Katre A, Cao D, Kadisha I, *et al*. Knockout of plasminogen activator inhibitor 1 gene reduces amyloid beta peptide burden in a mouse model of Alzheimer's disease. Neurobiol Aging 2011; 32: 1079-1089. http://dx.doi.org/10.1016/j.neurobiolaging.2009.06.003

[22] Akhter H, Katre A, Li L, Liu X, Liu RM. Therapeutic potential and anti-amyloidosis mechanisms of tert-butylhydroquinone for Alzheimer's disease. J Alzheimers Dis 2011; 26: 767-778.

[23] Samarakoon R, Dobberfuhl AD, Cooley C, Overstreet JM, Patel S, Goldschmeding R, Meldrum KK, Higgins PJ. Induction of renal fibrotic genes by TGF-β1 requires EGFR Activation, p53 and reactive oxygen species. Cell Signal 2013; 25: 2198-2209. http://dx.doi.org/10.1016/j.cellsig.2013.07.007

[24] Overstreet JM, Samarakoon R, Meldrum KK, Higgins PJ. Redox control of p53 in the Transcriptional regulation of TGF-β1 target genes through SMAD cooperativity. Cell Signal 2014; 26: 1427-1436. http://dx.doi.org/10.1016/j.cellsig.2014.02.017

[25] Barker R, Kehoe PG, Love S. Activators and inhibitors of the plasminogen system in Alzheimer's disease. J Cell Mol Med 2012; 16: 865-876. http://dx.doi.org/10.1111/j.1582-4934.2011.01394.x

[26] Jacobsen JS, Comery TA, Martone RL, Elokdah H, Crandall DL, Oganesian A, *et al*. Enhanced clearance of Aβ in brain by sustaining the plasmin proteolysis cascade. Proc Natl Acad Sci USA 2008; 105: 8754-8759. http://dx.doi.org/10.1073/pnas.0710823105

[27] Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH. Recovery of learning and memory is associated with chromatin remodeling. Nature 2007; 447: 178-182. http://dx.doi.org/10.1038/nature05772

[28] Sarma B, Singh N. Attenuation of vascular dementia by sodium butyrate in streptozotocin diabetic rats. Psychopharmacology 2011; 215: 677-687. http://dx.doi.org/10.1007/s00213-011-2164-0

[29] Dienel GA, Liu K, Cruz NF. Local uptake of ^{14}C-labeled acetate and buyrate in rat brain *in vivo* during spreading cortical depression. J Neuroscience Res 2011; 66: 812-820. http://dx.doi.org/10.1002/jnr.10063

[30] Kim HJ, Leeds P, Chuang D-M. The HDAC inhibitor, sodium butyrate, stimulates neurogenesis in the ischemic brain. J Neurochem 2009; 110: 1226-1240. http://dx.doi.org/10.1111/j.1471-4159.2009.06212.x

[31] Wyss-Coray T, lin C, Sanan DA, Mucke L, Mashiah E. Chronic overproduction of transforming growth factor-β1 by astrocytes promotes Alzheimer's disease-like microvascular degeneration in transgenic mice. Am J Pathol 2000; 156: 139-150. http://dx.doi.org/10.1016/S0002-9440(10)64713-X

[32] Lesné S, Docagne F, Gabriel C, Liot G, Lahiri DK, *et al*. Transforming growth factor-*β*1 potentiates amyloid-*β* generation in astrocytes and in transgenic mice. J Biol Chem 2003; 278: 18408-18418. http://dx.doi.org/10.1074/jbc.M300819200

[33] Lesné S, Blanchet S, Docagne F, Liot G, Plawinski L, *et al*. Transforming growth factor-*β*1-modulated cerebral gene expression. J Cereb Blood Flow Metabol 2002; 22: 1114-1123. http://dx.doi.org/10.1097/00004647-200209000-00009

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