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## Novel Combinatorial Therapeutic Targeting of PAI-1 (SERPINE1) Gene Expression in Alzheimer's Disease

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### Summary

Accumulation of neurotoxic amyloid peptides (A $\beta$ ) in the brain, generated by  $\beta$ -site proteolytic processing of the amyloid precursor protein (APP), is the hallmark pathophysiologic feature of Alzheimer's disease. The plasmin-activating cascade, in which urokinase (uPA) and tissue-type (tPA) plasminogen activators convert plasminogen to the broad-spectrum protease plasmin, appears to serve a protective, A $\beta$ -clearing, role in the central nervous system. Plasmin degrades A $\beta$  and catalyzes  $\alpha$ -site APP proteolysis generating nontoxic peptides. Plasmin activation in the brain is negatively regulated by the fast-acting clade E serine protease inhibitor (SERPIN) plasminogen activator inhibitor type-1 (PAI-1; SERPINE1) resulting in A $\beta$  accumulation. PAI-1 and its major physiological inducer TGF- $\beta$ 1, moreover, are both increased in Alzheimer's disease models and implicated in the etiology and progression of human neurodegenerative disorders. Current findings support the hypothesis that targeting of PAI-1 function (by small molecule drugs) and/or gene expression (by histone deacetylase inhibitors) may constitute a clinically-relevant molecular approach to the therapy of neurodegenerative diseases associated with increased PAI-1 levels.

### Plasmin-Activating System in Alzheimer's Disease

Aggregated  $\beta$ -amyloid peptide plaques accumulate in specific areas of the brain in patients with Alzheimer's disease (AD) by proteolytic processing of the single-pass transmembrane APP [1]. These deposits trigger prolonged inflammation, neuronal death, and progressive cognitive decline [2]. A $\beta$  peptides are produced by aspartic protease (BACE)-induced  $\beta$ -site cleavage of APP creating a membrane-bound COOH-terminal C99 fragment followed by proteolysis (involving presenilin and nicastrin) at the C99 transmembrane-localized  $\gamma$  position [3–5]. There is also an alternative APP processing pathway in which a membrane-proximal ( $\alpha$ -site) cleavage, by matrix metalloproteinases (TACE, ADAM 10), replaces  $\beta$  position utilization producing a membrane-anchored C83 fragment. Subsequent  $\gamma$  processing of the C83 product generates the nontoxic p3 peptide [3,6].

Among other targets, the broad-spectrum protease plasmin also degrades A $\beta$  [7–9] and plasmin activation decreases A $\beta$  peptide levels [10]. Plasmin-mediated proteolysis of APP, moreover, involves the  $\alpha$ -site (either as a direct or indirect target) resulting in decreased A $\beta$  production, suggesting a protective role for the plasmin cascade in the central nervous system. Indeed, plasmin levels in the brains of AD patients are considerably reduced [10]

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supporting a causal relationship between deficient activity of the plasmin-generating proteolytic system and accumulation of A $\beta$  in the progression of AD (Figure 1).

## Therapeutic Approaches

Several members of the SERPIN superfamily exhibit cell-type neurotrophic, neuroprotective, or neuropathophysiologic activities [11]. These include SERPINF1, SERPINI1 (neuroserpin), SERPINE1 (PAI-1), SERPINE2 (nexin-1), and SERPINA3 [11]. PAI-1 (SERPINE1), in particular, has multifunctional roles in the central nervous system as it both maintains neuronal cellular structure and initiates signaling through the mitogen-activated protein kinase pathway [12]. Significantly increased PAI-1 immunoreactivity in the central nervous system of AD patients was associated with development of senile plaques and ghost tangle structures [13] consistent with the earlier colocalization of plasminogen activators and PAI-1 in plaque structures [14] which are sites of sustained inflammation [15]. Tg2576 and TgCRN8 transgenic mice, engineered to express brain-targeted Swedish mutant A $\beta$  and the double Swedish/V717F mutant A $\beta$ , respectively, exhibit age-dependent A $\beta$  plaque development as well as cognitive deficiencies [16]. tPA activity in these mice was significantly decreased specifically in the hippocampus and amygdala correlating with corresponding regional increases in brain PAI-1 expression [17]. Since direct A $\beta$  peptide injection increased PAI-1 expression and A $\beta$  removal from the hippocampal region required both tPA and plasminogen, a functional tPA-plasmin axis appears required for A $\beta$  clearance [17]. While PAI-1 may be neuroprotective in specific acute injury settings (e.g., tPA-triggered neuronal apoptosis) [18,19], chronically elevated PAI-1 levels likely promote A $\beta$  accumulation by inhibiting plasmin-dependent degradation. Genetic evidence clearly indicates that brain PAI-1 expression is increased in A $\beta$  precursor protein presenilin 1 (APP/PS1) mice as well as in AD patients [20] while PAI-1-deficiency in an APP/PS1 transgenic background reduces amyloid accumulation. These findings have therapeutic implications as a small molecule inhibitor of PAI-1 activity (PAZ-417) partially blocks amyloid deposition in a mouse model of AD. The mechanism, perhaps as expected, suggests that PAI-1 inhibition stimulates tPA/plasmin activity, decreasing brain A $\beta$  levels and reverses cognitive deficits [21]. The development of pharmacologic strategies to prevent and therapeutically manage patients at risk for, and who present with, AD by inhibiting the function of a key contributor (PAI-1) to disease progression has significant translational relevance. Indeed, histone deacetylase inhibitors (HDACi) have potential promise as a therapy for neurodegenerative disease [22]. Sodium butyrate (NaB), a broad-spectrum HDACi, attenuated streptozotocin-induced endothelial dysfunction and improved learning and memory (Morris water maze test) in rats [23]. Butyrate localizes to the cerebral cortex in KCl-induced spreading cortical depression [24] while NaB (as well as TSA and valproic acid) are neuroprotective in the ischemic brain [25]. Most importantly, several HDACi (NaB, TSA, SAHA and to a lesser extent sirtinol) effectively reduced TGF- $\beta$ 1-induced PAI-1 expression [19] (Figure 2). This has translational implications as brain TGF- $\beta$ 1 levels are elevated in the onset and progression of Parkinson's disease, AD and stroke [19]. Increased expression of TGF- $\beta$ 1 correlates with A $\beta$  angiopathy and transgenic mice that overexpress TGF- $\beta$  in astrocytes exhibit early onset A $\beta$  deposition [26]. TGF- $\beta$ 1, moreover, induces astrocyte APP expression through a TGF- $\beta$ 1-responsive element in the APP promoter while A $\beta$  production was enhanced by TGF- $\beta$ 1 signaling [27]. The coordinate overexpression of PAI-1 and increased A $\beta$  generation in response to elevated TGF- $\beta$ 1 in AD patients is likely to predispose to disease progression [28]. Collectively, these findings raise the possibility that targeting TGF- $\beta$ 1-inducible genes (e.g., PAI-1, APP) will provide a 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.

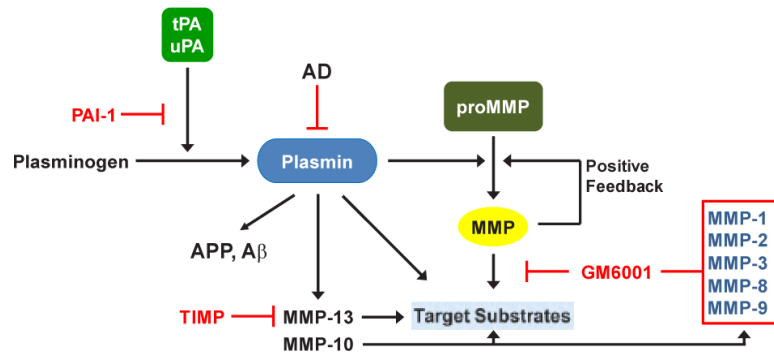
## Acknowledgments

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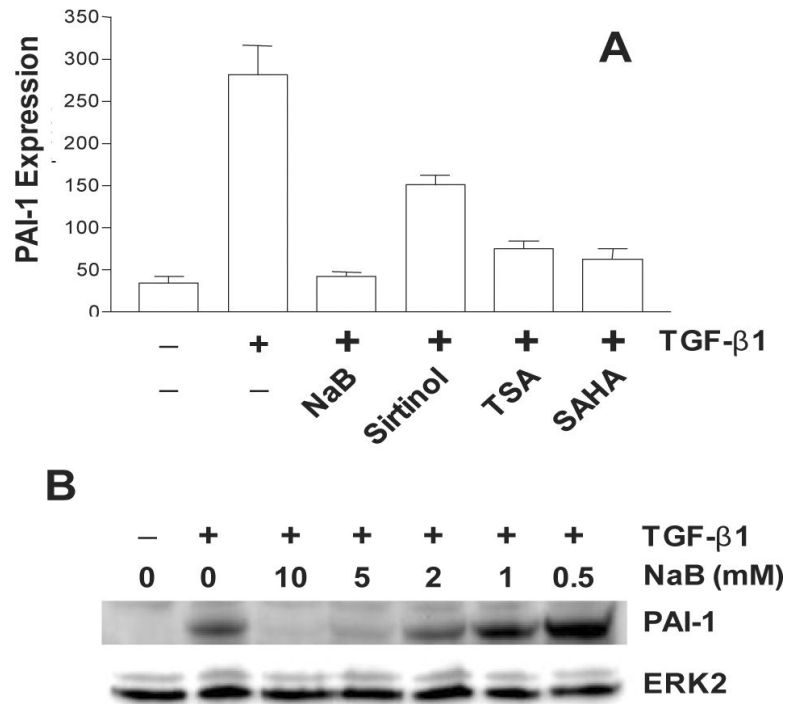
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**Figure 1.**

tPA and uPA convert plasminogen to the active protease plasmin that, in turn, degrades target substrates (e.g., APP, A $\beta$ ) directly as well as indirectly through downstream activation of matrix metalloproteinases (MMPs). Inhibition of MMP activity with tissue inhibitor of metalloproteinases (TIMP) or GM6001 has confirmed their participation in plasmin-initiated proteolysis. This cascade is effectively attenuated by expression of PAI-1 which blocks tPA and uPA catalysis inhibiting plasmin generation.



**Figure 2.** Effect of the histone deacetylase inhibitors sodium butyrate (NaB), sirtinol, trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) on TGF- $\beta$ 1-induced PAI-1 expression (A). Data plotted is a summary of western blot analyses. Preincubation with NaB concentrations of 5–10 mM completely ablates TGF- $\beta$ 1-stimulated PAI-1 expression (B).