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Review Article

The TGF-β1/Upstream Stimulatory Factor-Regulated PAI-1 Gene: Potential Involvement and a Therapeutic Target in Alzheimer's Disease

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Amyloid peptide (Aβ) aggregates, derived from initial β-site proteolytic processing of the amyloid precursor protein (APP), accumulate in the brains of Alzheimer's disease patients. The plasmin-generating cascade appears to serve a protective role in the central nervous system since plasmin-mediated proteolysis of APP utilizes the α site, eventually generating nontoxic peptides, and plasmin also degrades Aβ. The conversion of plasminogen to plasmin by tissue-type plasminogen activator in the brain is negatively regulated by plasminogen activator inhibitor type-1 (PAI-1) resulting in attenuation of plasmin-dependent substrate degradation with resultant accumulation of Aβ. PAI-1 and its major physiological inducer TGF-β1, moreover, are increased in models of Alzheimer's disease and have been implicated in the etiology and progression of human neurodegenerative disorders. This review highlights the potential role of PAI-1 and TGF-β1 in this process. Current molecular events associated with TGF-β1-induced PAI-1 transcription are presented with particular relevance to potential targeting of PAI-1 gene expression as a molecular approach to the therapy of neurodegenerative diseases associated with increased PAI-1 expression such as Alzheimer's disease.

INTRODUCTION

In patients with Alzheimer's disease (AD), plaques comprised of aggregated β-amyloid peptides (Aβ) accumulate in specific areas of the brain as a consequence of the proteolytic processing of the single-pass transmembrane amyloid precursor protein (APP) [1]. These Aβ deposits trigger prolonged inflammation, neuronal death, and progressive cognitive decline [2]. Aβ peptides are derived from APP by β-site cleavage by an aspartic protease (BACE) producing a membrane-bound COOH-terminal C99 fragment followed by a complex proteolytic event (involving presenilin and nicastrin) at the C99 transmembrane-localized γ position [3–5]. An alternative APP processing pathway also exists in which membrane-proximal (α-site) cleavage by matrix metalloproteinases (TACE, ADAM 10) replaces β position utilization producing a membrane-anchored C83 fragment. Subsequent γ-site processing of the C83 product results in generation of the nontoxic p3 peptide [3, 6].

The broad-spectrum protease plasmin also degrades Aβ [7–9] and activation of plasmin decreases Aβ peptide levels [10]. Plasmin-mediated proteolysis of APP, moreover, appears to involve the α site (either as a direct or indirect target) resulting in decreased Aβ production, thus 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] further supporting a causal relationship between deficient activity of the plasmin-generating proteolytic system and accumulation of Aβ in the progression of AD.

PLASMIN-ACTIVATING SYSTEM IN ALZHEIMER'S DISEASE

Several members of the serine protease inhibitor (SERPIN) superfamily exhibit neurotrophic, neuroprotective, or neuropathophysiologic activities depending on the specific cell type and pathways involved [11]. These include SERPINF1, SERPINI1 (neuroserpin), SERPINE1 (plasminogen activator inhibitor type-1; PAI-1), SERPINE2 (nexin-1), and SERPINA3 [11]. PAI-1, in particular, has multifunctional roles in the central nervous system as it both maintains neuronal cellular structure and initiates signaling through the ERK pathway [12]. PAI-1 directly influences the plasmin-dependent pericellular proteolytic cascade by regulating the conversion of plasminogen to plasmin by urokinase- and tissue-type plasminogen activators (uPA/tPA) (Figure 1).

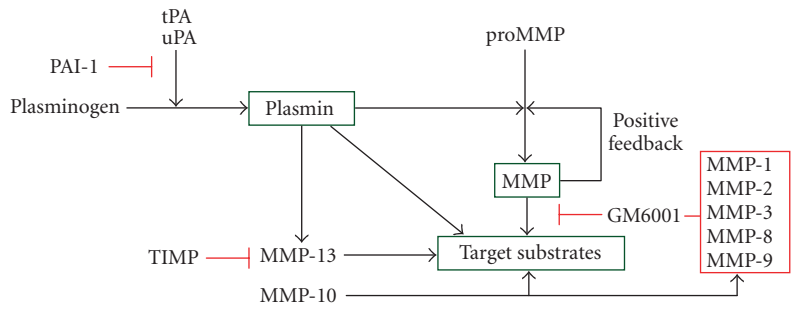

Figure 1: tPA and uPA convert plasminogen to the active, broad-spectrum, protease plasmin both at the cell surface and in the immediate pericellular space. Plasmin, in turn, degrades target substrates (eg, APP, *Aβ*) directly as well as indirectly through downstream activation of matrix metalloproteinases (MMPs). Inhibition of MMP activity (GM6001, TIMP) has confirmed their participation in plasmin-initiated proteolysis. Most importantly, this cascade is effectively attenuated by overexpression (or exogenous addition) or PAI-1 which blocks tPA and uPA catalysis inhibiting, thereby, plasmin generation.

PAI-1 immunoreactivity in the central nervous system of AD patients was associated with senile plaques and ghost tangle structures [13] consistent with the earlier colocalization of PA and PAI-1 in plaque structures [14] which are sites of sustained inflammation [15]. Recent findings in Tg2576 and TgCRN8 transgenic mice, engineered to express brain-targeted Swedish mutant Aβ and the double Swedish/V717F mutant Aβ, respectively, under control of the hamster prion promoter and exhibit age-dependent Aβ plaque development (at 12 and 3 months, resp) as well as cognitive deficiencies [16], established that tPA activity was significantly decreased compared to controls [17]. This decline correlated with corresponding increases in PAI-1 expression specifically in areas of the brain where tPA activity was reduced (hippocampus, amygdala). Since direct Aβ peptide injection increased PAI-1 expression and whereas Aβ removal from the hippocampal region required both tPA and plasminogen, it appears that a functional tPA-plasmin axis is required for Aβ clearance [17]. While PAI-1 may be neuroprotective in specific acute injury settings (eg, tPA-triggered neuronal apoptosis) [18], chronically elevated PAI-1 levels likely promote Aβ accumulation by inhibiting plasmin-dependent degradation (Figure 1).

CONTROLS ON TGF-β1 TARGET GENES: THE PAI-1 MODEL

Several reports described elevated TGF-β1 levels in brain biopsies from patients with Parkinson's disease, AD, and stroke [20–22]. This growth factor is likely to influence the onset and progression of AD at several levels. Increased brain expression of TGF-β1 correlates with Aβ angiopathy, and transgenic mice that overexpress TGF-β1 in astrocytes exhibit early onset Aβ deposition [23]. TGF-β1, moreover, induces astrocyte APP expression through a TGF-β1-responsive AGAC Smad-binding element in the APP promoter; subsequent Aβ production, moreover, was enhanced by TGF-β1 signaling [24]. Since the PAI-1 gene is also transcriptionally upregulated by TGF-β1 [19, 25], the coordinate overexpression of PAI-1 and increased Aβ generation in response to elevated TGF-β1 in the brains of AD patients may dispose to disease progression [26]. Collectively, these findings raise the possibility that targeting TGF-β1-inducible genes (eg, PAI-1, APP) may have therapeutic benefit in the setting of AD.

The regulation of TGF-β1-activated genes (ie, PAI-1) is largely transcriptional [19, 25, 27, 28] with the PAI-1 gene subject to complex combinatorial expression controls involving the major transcription effectors p53, Sp1, and members of the MYC family [19, 29, 30]. Prominent TGF-β-response elements in the human PAI-1 promoter include the hexanucleotide E box motif (5′-CACGTG-3′; as in the PE1, PE2, HRE-2 sites) and closely related sequences recognized by the basic helix-loop-helix/leucine zipper (bHLH-LZ) transcription factors of the MYC family (eg, MYC, MAX, TFE3, USF-1, and USF-2) [31–36]. This E box element likely functions, therefore, as a "platform" for recruitment of both positive and negative regulators of PAI-1 expression [37–39]. Recent UV crosslinking and tethered DNA affinity chromatographic analyses identified the bHLH-LZ protein upstream stimulatory factor-1 (USF-1) as a major PAI-1 E box-recognition factor [40]. Specific E box mutations that ablate USF-1 binding to a PAI-1 target deoxyoligonucleotide probe (CA → TC) effectively attenuated TGF-β1-stimulated PAI-1 promoter-driven CAT reporter activity [36]. The human PAI-1 promoter, however, harbors several additional TGF-β-responsive elements, including three E box-adjoining Smad sequences located just 5′ of the PE2 site [32, 33, 35, 41]. Since an engineered two-base-pair mutation CA**CG**TG → CA**AT**TG in a serum-responsive PAI-1 E box attenuated growth state as well as TGF-β1-dependent transcription [36], this same dinucleotide substitution was incorporated into a luciferase reporter construct bearing the immediate 806 base pairs of the human PAI-1 5′ upstream region. Initial truncation approaches did, in fact, confirm that a major (albeit not the only) TGF-β1-responsive element resided within the most proximal 606 base pairs of the human PAI-1 promoter [19]. Specific disruption of the PE2 region E box element by site-directed mutagenesis significantly attenuated TGF-β1-mediated PAI-1 transcription (Figure 2)

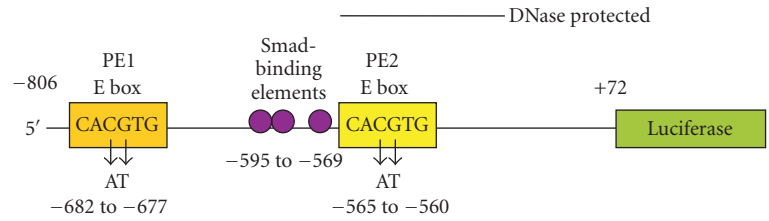

Figure 2: Topography of the PAI-1p806-Luc reporter construct illustrating the two (PE1 and PE2) E box sequences. Site-directed mutagenesis and luciferase assays clearly indicated that an intact E box at the PE2 site is required for maximal TGF-β1-induced PAI-1 transcription in human epithelial cells [19].

[19]. The consequences of USF binding to the PAI-1 PE2 E box site may be more complex, however, than simple motif occupancy. Indeed, certain E box-recognition factors including USF-1 and USF-2 effectively induce DNA bending. Cooperative interactions between Sp1 and/or p53 with USF proteins, for example, may be dependent on USF-initiated modifications to DNA conformation allowing distally spaced factors important in expression control to interact with resulting effects on PAI-1 transcription [42].

SEQUENCE REQUIREMENTS FOR USF OCCUPANCY OF THE PE2 REGION E BOX MOTIF

Chromatin immunoprecipitation recently confirmed that the PAI-1 PE2 E box site is an *in vivo* USF target motif [38]. Since an intact consensus PE2 region E box sequence is necessary for a maximal transcriptional response to growth factors [19], it was important to identify any additional requirements for PE2 E box-occupancy that might influence site residence including the Smad-binding AGAC elements implicated in TGF-β1-dependent APP expression [24]. PE1 and PE2 probes recognition appeared dependent solely on an intact 5′-CACGTG-3′ motif since nuclear factor binding to individual PE1 and PE2 target constructs was successfully blocked by short double-stranded deoxyoligonucleotides containing a consensus E box flanked by non-PAI-1 sequences whereas a mutant E box (5′-CAATTG-3′) "bait" failed to compete [19]. It was important, however, to confirm these results using site-specific mutants within the context of native PAI-1 promoter sequences (eg, the PE2 region backbone) in order to assess the potential contributions of the Smad-binding elements, E box flanking nucleotides (such as the AAT trinucleotide "spacer" between the PE2 E box and the first upstream Smad site), and the CACGTG motif to nuclear protein binding (Figure 2). A recent study established that the major protein/DNA interactions in the PE2 segment were, in fact, E box-dependent and did not require accessory sites since mutation of all three Smad-binding sites (AGAC → CTTG) or removal of the ATT spacer did not affect USF occupancy of the PE2 region E box [19]. While the CACGTG "core" is a target for occupancy by at least seven members of the bHLH-LZ transcription factor family (USF-1, USF-2, c-MYC, MAX, TFE3, TFEB, TFII-I), USF proteins have a preference for C or T at the −4 position in the presence of $MgCl_2$ [43]. Indeed, the human PAI-1 gene has a T at the −4 site of the PE2 region E box as well as a purine at +4 and −5 and a pyrimidine at +5 ($A_{-5}T_{-4}C_{-3}A_{-2}C_{-1}G_{+1}T_{+2}G_{+3}G_{+4}C_{+5}$), all of which facilitate USF binding [43]. Chromatin immunoprecipitation of the PE2 region E box site in the human PAI-1 gene, moreover, indicated a dynamic occupancy by USF subtypes (USF-1 versus USF-2) as a function of growth state [44]. This motif was clearly a platform for USF-1 binding in quiescent cells. An exchange of PE2 E box USF-1 homodimers with USF-2 homo- or USF-1/USF-2 heterodimers, furthermore, closely correlated with PAI-1 gene activation. This switch may well determine the transcriptional status of the PAI-1 gene in quiescent versus growth factor-stimulated culture conditions [38, 45]. Site occupancy and transcriptional activity additionally require conservation of the PE2 core E box structure as the CACGTG → CACGGA and TCCGTG dinucleotide substitutions (in the rat gene) [36] and a CACGTG → CAATTG or TCCGTG replacement (in the human gene), with retention of PAI-1 flanking sequences, resulted in loss of both competitive binding and growth factor-dependent reporter activity [19, 44]. The CACGTG → TCCGTG mutation is particularly relevant since bHLH proteins with E box-recognition activity have a conserved glutamate important for interaction with the first two nucleotides (CA) in the E box motif [46]. These data are also consistent with the known hexanucleotide preference (CACGTG or CACATG) of USF proteins [39, 47, 48]. To further dissect the role of USF in TGFβ1-mediated PAI-1 transcription *in vivo*, a dominant-negative USF construct (A-USF) was implemented for molecular genetic targeting [19]. A-USF effectively titers away functional USF proteins by forming highly stable interactions with native USF proteins; such USF/A-USF heterodimers, however, are unable to bind DNA due to replacement (in the A-USF construct) of the basic DNA-binding residues with an acidic domain [49]. A-USF transfection effectively attenuated TGF-β1-induced PAI-1 expression establishing the importance of USF family transcription factors in PAI-1 gene control [19, 50, 51].

MAPPING THE TGF-β1-INDUCED PAI-1 SIGNALING AND TRANSCRIPTIONAL CONTROL NETWORKS: OPPORTUNITIES FOR THERAPEUTIC INTERVENTION

The molecular mechanisms associated with the TGF-β1-initiated E box-dependent PAI-1 gene control and the collateral Smad-mediated APP induction/TGF-β-directed Aβ

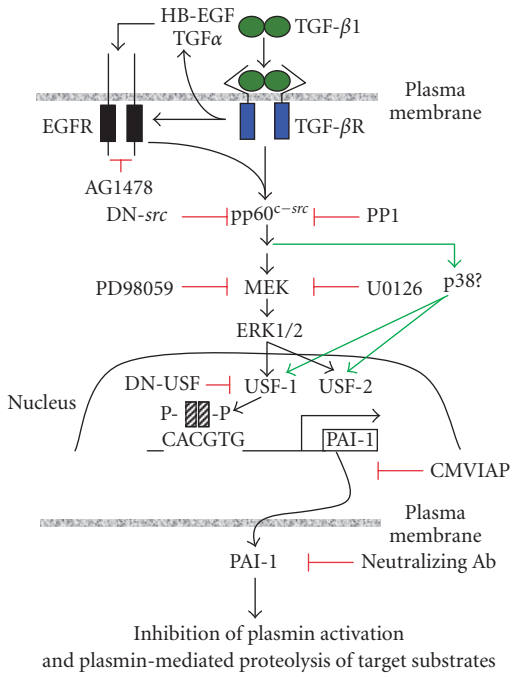

FIGURE 3: Pathways involved in the regulation of PAI-1 expression and function in response to TGF-β1 stimulation. Positve influences are depicted in black arrows; effective inhibitors defined pharmacologically or by use of dominant-negative constructs (DN) are highlighted in red (detailed in [50]). CMVIAP = PAI-1 antisense expression vector.

processing in specific central nervous system cell types remain to be clarified. The available data, however, clearly suggest that these two responses to TGF-β1 are linked in the pathophysiology of human neurodegenerative disease. It has become apparent, moreover, that PAI-1 overexpression is a likely major contributory if not a causative event in AD progression. Our current understanding of the pathways utilized by the TGF-β1 to stimulate the PAI-1 transcription (*summarized in Figure 3*) indicate that this growth factor activates a kinase cascade, at least partially as a function of epidermal growth factor receptor mobilization (either through the release of the appropriate ligands or the direct receptor transactivation), involving MEK, ERK1/2, and perhaps p38 [49–51]. Pharmacological approaches, use of dominant-negative constructs, and kinase assays suggest that *src* family kinases and *ras* GTPase are upstream of MEK-ERK-p38 in this model of induced PAI-1 expression [50, 51]. MAP kinases, in turn, interact with nuclear transcription factors including members of the USF family that, once phosphorylated, bind as dimers to E box motifs in the PAI-1 promoter to modulate gene expression [19, 26, 38, 40, 50, 51]. Genetic perturbation of PAI-1 synthesis in specific areas of the brain (with dominant-negative USF or PAI-1 antisense vectors) or delivery of PAI-1 neutralizing antibodies may effectively stimulate uPA- and/or plasmin-dependent target substrate degradation (eg, Aβ) or at least attenuate the rate of Aβ accumulation (Figures 1 and 3). The continued identification of regulatory points in the PAI-1 expression control network (Figure 3) and recent identification of TGF-β1-response sites (as well as the involved nuclear factors) in the APP and PAI-1 promoters [19, 38, 40, 52] may provide new molecular targets for the therapy of neurodegenerative syndromes associated with PAI-1 upregulation. Indeed, specific SERPINS (including PAI-1) have already been suggested as potential novel therapeutic targets for stroke and cerebral ischemia [12]. The TGF-β1 gene, furthermore, is also USF-regulated [53] suggesting that interference with USF-dependent transcriptional events may have widespread therapeutic implications.

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