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PAI-1 Promoter-specific Oligonucleotide Decoys: Transcription Factor "Bates" and Potential Utility as Wound Healing Therapeutics

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Introduction

Double-strand Oligo DeoxyNucleotides (ODNs) or hairpin ODNs that mimic binding sites for transcription factors are effective cis-element decoys. These short (15-30 mer) platforms are designed to contain protein-binding consensus recognition sites or modified sequences permissive for stronger protein–DNA interactions [1]. Transfected decoys bind construct-specific transcriptional effectors effectively titrating trans-acting factors from their target promoters. Sp1, AP-1, STAT3, and Ets-1 decoys inhibit expression of cancer-associated genes as well as attenuate tumor cell growth and metastasis [2-5]. Despite similarity in the consensus binding sequences between STAT1 and STAT3, a decoy was developed that specifically sequesters STAT3 but not STAT1 [6], demonstrating the feasibility of differential discrimination among closely-related transcription factors. While decoy delivery in vivo is major challenge for clinical applications, recent findings highlight the utility and advantages of ODN technologies in animal model systems [7,8] including specific modifications (i.e., incorporation of phosphorothioate groups) to increase stability. Recent advances include development of circularized or "block" decoys which are chimeric constructs that target multiple regulatory elements [9].

The USF/PAI-1 Axis as a Model

Upstream stimulatory factor-1 and -2 (USF1/2) are basic helix-loop-helix/leucine zipper (bHLHLZ) E box-binding transcription factors the functions of which are dependent on site-specific phosphorylation. Dimer composition and recruited co-factors dictate target gene expression [10-12]. USF1/2 regulate growth state-dependent transcription of plasminogen activator inhibitor-1 (PAI-1, SERPINE1) [11], a major TGF-β1-responsive and p53 target gene [13,14]. PAI-1 controls pericellular plasmin generation and is a prominent member of the "wound-response" transcriptome [15,16]. PAI-1 is required for TGF-β1-stimulated keratinocyte planar migration and stromal barrier invasion likely via LRP1-mediated engagement of the Jak/Stat pathway [14,17]. This SERPIN is a non-structural Extracellular Matrix (ECM)- associated (matricellular) protein [18] that promotes a mesenchymal-to-amoeboid transition with activation of a intracellular signaling cascade required for efficient 3-D "stromal" migration [19,20]. The consistent association of PAI-1 expression with the global program of tissue injury [21] suggests that this SERPIN integrates cycles of cell-to-substrate adhesion/dis-adhesion with repair "scaffold" remodeling to meet the requirements for effective cellular migration [22]. A recent assessment of the transcriptional signature among the spectrum of wound healing responses (i.e., non-scarring regenerative repair, scar formation, chronic non-healing injuries) clearly indicated that PAI-1 partitioned to the dysfunctional healing gene set repertoire [23] emphasizing its candidacy as a translationally-important target in the context of tissue repair anomalies associated with deficient or excessive PAI-1 levels [21].

USF Involvement in PAI-1 Transcription

PAI-1 transcription is an early event in serum-stimulated quiescent (G_0) keratinocytes and fibroblasts. Chromatin ImmunoPrecipiation (ChIP) confirmed that PAI-1 expression occurs prior to G_1 entry and involves a USF subtype switch (USF1→USF2) at the PE1/PE2 site E box motifs (5'-CACGTG-3') in the PF1 region (nucleotides -794 to -532) of the PAI-1 promoter [11]. The PE2 E box, moreover, is 5'-flanked by three SMAD-binding sites although mobility shift studies indicated that these AGAC sequences are not likely required for USF occupancy of a PE2 region E box target [11,24]. While USF1/2 binding to PE1/PE2 targets in serum-stimulated cells was independent of growth state (Figure 1), ChIP analysis confirmed USF1→USF2 dimer replacement at the critical PE2 E box motif reflected induced PAI-1 expression indicating that promoter occupancy was distinct from simple probe recognition [11].

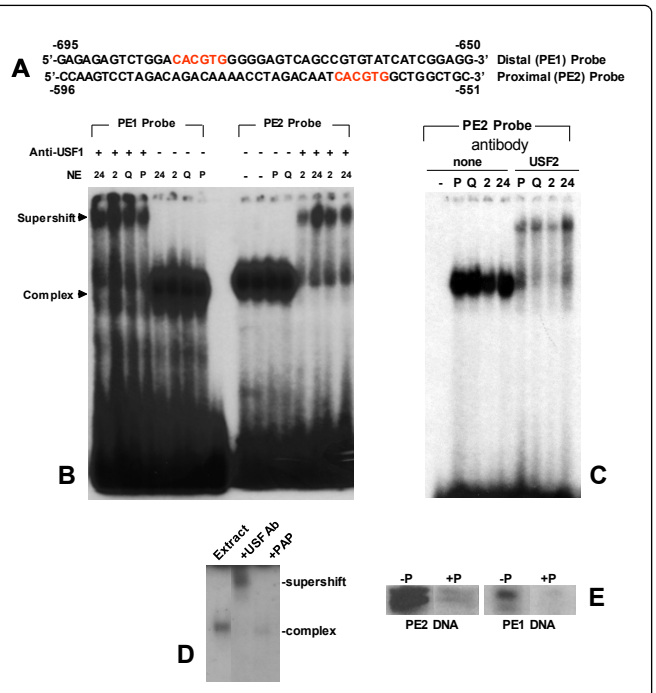

Figure 1: USF1/2 bind to PE1 and PE2 PAI-1 promoter region DNA target probes.

Double-stranded ^{32}P-labeled PE1 or PE2 constructs (A; only top strand is illustrated for both probes) were incubated with nuclear extracts from proliferating (P), quiescent (Q) and 2 as well as 24 hour (2,24) serum-stimulated human HaCaT II-4 keratinocytes. Antibodies to the E box-binding USF1 and USF2 were added where indicated and protein-probe complexes separated on non-denaturing polyacrylamide gels. (–) =absence of nuclear extract; none=no antibody added. Positions of the original protein-probe complex and the USF antibody-induced supershift are indicated with arrows (B-D). PE1/PE2 region probe recognition required phosphorylation as extract pretreatment with Potato Acid Phosphatase (PAP) inhibited USF-DNA complex formation as determined by standard electrophoretic mobility shift (D) and biotinylated oligonucleotide pull-down (E) assays.

P in (E) = PAP

APE2 Region Decoy Effectively Attenuates TGF-β1-Induced PAI-1 Expression

PAI-1 transcriptional activation has important phenotypic consequences. Several SERPINS (including SERPINE1 [PAI-1], SERPINB1, SERPINB2) are prominent members of the "tissue repair" transcriptome where they function in the integrated control of focalized extracellular matrix restructuring, cell-to-substrate adhesion/detachment, migration and proliferation [14,16,17,21,22]. PAI-1 limits urokinase (uPA)-mediated pericellular plasmin generation to maintain a supporting "scaffold" for cell movement [22] while also regulating uPA-dependent growth factor activation attenuating, thereby, the associated proliferative response [13]. Using the sequence restraints for PAI-1 promoter-driven reporter activation [25] and DNA binding [11], a double-stranded 45-bp PE2 DNA construct was designed based on the requirements for an intact CACGTG motif for probe recognition by USF [11,24]. Transfection of these double-stranded USF binding, "decoys" effectively reduced both serum- and TGF-β1-induced PAI-1 transcript levels in HaCaT II-4 keratinocytes (Figure 2). This finding has important translational implications. A recent review [21] highlighted the association of elevated PAI-1 with healing anomalies including keloid development and hypertrophic scarring while an increased uPA/PAI-1 ratio, favoring high uPA activity, is characteristic of chronic ulcers. Excessive scarring at various tissue sites consistently reflects augmented PAI-1 expression, a prominent TGF-β1 target, further underscoring the clinical potential in the development of PAI-1 decoys (Figure 2). Indeed, PAI-1 small interfering RNAs effectively attenuate collagen levels in keloids and implantation of polyvinyl alcohol sponges into PAI-1–/– mice resulted in a significantly reduced fibrotic response. PAI-1 deficiency promotes an accelerated skin wound healing response while plasminogen stimulated the repair of acute and diabetic wounds [reviewed in 21]. More recently, small molecule PAI-1 functional inhibitors demonstrated efficacy as anti-fibrotic agents in experimental models of TGF-β1–induced pulmonary fibrosis and skeletal muscle repair. The present findings suggest that PAI-1 PE2 region decoys may find utility as novel, expression-regulating therapeutics.

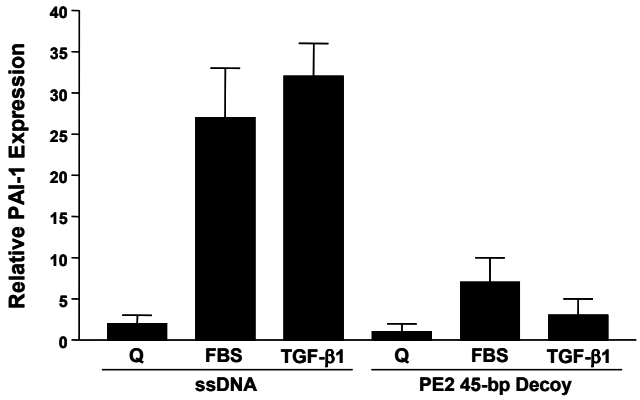

Figure 2: Transfection of a 45-bp double-stranded PE2 decoy construct attenuates PAI-1 expression in TGF-β1-stimulated cells. Transfection of HaCaT II-4 keratinocytes cells with a double-stranded USF-binding 45-bp PE2 region decoy construct markedly decreased the levels of both serum- and TGF-β1-induced mRNA PAI-1 transcripts. Graphed data is the mean+ SD of 3 independent experiments. sDNA=sheared, double-stranded, control DNA.

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References

1. Mann MJ (2005) Transcription factor decoys: a new model for disease intervention. Ann N Y Acad Sci 1058: 128-139.
2. Ishibashi H, Nakagawa K, Onimaru M, Castellanous EJ, Kaneda Y, et al. (2000) Sp1 decoy transfected to carcinoma cells suppresses the expression of vascular endothelial growth factor, transformation growth factor 1, and tissue factor and also cell growth and invasion activities. Cancer Res 60:6531–6536.
3. Shiratsuchi T, Ishibashi H, Shirasuna K (2002) Inhibition of epidermal growth factor-induced invasion by dexamethasone and AP-1 decoy in human squamous cell carcinoma cell lines. J Cell Physiol 193: 340-348.
4. Leong PL, Andrews GA, Johnson DE, Dyer KF, Xi S, et al. (2003) Targeted inhibition of Stat3 with a decoy oligonucleotide abrogates head and neck cancer cell growth. Proc Natl Acad Sci U S A 100: 4138-4143.
5. Taniguchi H, Fujiwara Y, Doki Y, Sugita Y, Sohma I, et al. (2007) Gene therapy using ets-1 transcription factor decoy for peritoneal dissemination of gastric cancer. Int J Cancer 121: 1609-1617.
6. Souissi I, Ladam P, Cognet JAH, Le Coquil S, Varin-Blank N, et al. (2012) A STAT3-inhibitory hairpin decoy oligodeoxynucleotide discriminates between STAT1 and STAT3 and induces death in a human colon carcinoma cell line. Mol Cancer 11:1476-4598.
7. Koppikar P, Bromberg J (2012) STATe-of-the-art approach: using oligonucleotide decoys to target the "undruggable". Cancer Discov 2: 670-672.
8. Yan C, Higgins PJ (2013) Drugging the undruggable: transcription therapy for cancer. Biochim Biophys Acta 1835: 76-85.
9. Brown AJ, Mainwaring DO, Sweeney B, James DC (2013) Block decoys: transcription-factor decoys designed for in vitro gene regulation studies. Anal Biochem 443: 205-210.

10. Galibert MD, Carreira S, Goding CR (2001) The Usf-1 transcription factor is a novel target for the stress-responsive p38 kinase and mediates UV-induced Tyrosinase expression. EMBO J 20: 5022-5031.
11. Qi L, Allen RR, Lu Q, Higgins CE, Garone R, et al. (2006) PAI-1 transcriptional regulation during the G0 --> G1 transition in human epidermal keratinocytes. J Cell Biochem 99: 495-507.
12. Pawlus MR, Wang L, Murakami A, Dai G, Hu CJ (2013) STAT3 or USF2 contributes to HIF target gene specificity. PLoS One 8: e72358.
13. Kortlever RM, Higgins PJ, Bernards R (2006) Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. Nat Cell Biol 8: 877-884.
14. Freytag J, Wilkins-Port CE, Higgins CE, Higgins SP, Samarakoon R, et al. (2010) PAI-1 mediates the TGF-beta1+EGF-induced "scatter" response in transformed human keratinocytes. J Invest Dermatol 130: 2179-2190.
15. Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, et al. (1999) The transcriptional program in the response of human fibroblasts to serum. Science 283: 83-87.
16. Qi L, Higgins SP, Lu Q, Samarakoon R, Wilkins-Port CE, et al. (2008) SERPINE1 (PAI-1) is a prominent member of the early G0 --> G1 transition "wound repair" transcriptome in p53 mutant human keratinocytes. J Invest Dermatol 128: 749-753.
17. Degryse B, Neels JG, Czekay RP, Aertgeerts K, Kamikubo Y, et al. (2004) The low density lipoprotein receptor-related protein is a motogenic receptor for plasminogen activator inhibitor-1. J Biol Chem 279: 22595-22604.
18. Maquerlot F, Galiacy S, Malo M, Guignabert C, Lawrence DA, et al. (2006) Dual role for plasminogen activator inhibitor type 1 as soluble and as matricellular regulator of epithelial alveolar cell wound healing. Am J Pathol 169: 1624-1632.
19. Cartier-Michaud A, Malo M, Charrière-Bertrand C, Gadea G, Anguille C, et al. (2012) Matrix-bound PAI-1 supports cell blebbing via RhoA/ROCK1 signaling. PLoS One 7: e32204.
20. Grahovac J, Wells A (2014) Matrikine and matricellular regulators of EGF receptor signaling on cancer cell migration and invasion. Lab Invest 94: 31-40.
21. Simone TM, Higgins CE, Czekay RP, Law BK, Higgins SP, et al. (2014) SERPINE1: A Molecular Switch in the Proliferation-Migration Dichotomy in Wound-"Activated" Keratinocytes. Adv Wound Care (New Rochelle) 3: 281-290.
22. Czekay RP, Wilkins-Port CE, Higgins SP, Freytag J, Overstreet JM, et al. (2011) PAI-1: An Integrator of Cell Signaling and Migration. Int J Cell Biol 2011: 562481.
23. Peake MA, Caley M, Giles PJ, Wall I, Enoch S, et al. (2014) Identification of a transcriptional signature for the wound healing continuum. Wound Repair Regen 22: 399-405.
24. Allen RR, Qi L, Higgins PJ (2005) Upstream stimulatory factor regulates E box-dependent PAI-1 transcription in human epidermal keratinocytes. J Cell Physiol 203: 156-165.
25. Qi L, Higgins CE, Higgins SP, Law BK, Simone TM, et al. (2014) The Basic Helix-Loop-Helix/Leucine Zipper Transcription Factor USF2 Integrates Serum-Induced PAI-1 Expression and Keratinocyte Growth. J Cell Biochem 115: 1840-1847.