The Transcriptional Signatures of Cells from the Human Peyronie's Disease Plaque and the Ability of These Cells to Generate a Plaque in a Rat Model Suggest Potential Therapeutic Targets

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DOI: 10.1111/jsm.12760

ABSTRACT

Introduction. The success of medical therapies for Peyronie's disease (PD) has not been optimal, possibly because many of them went directly to clinical application without sufficient preclinical scientific research. Previous studies revealed cellular and molecular pathways involved in the formation of the PD plaque and in particular the role of the myofibroblast.

Aims. The current work aimed to determine under normal and fibrotic conditions what differentiates PD cells from tunica albuginea (TA) and corpora cavernosa (CC) cells by defining their global transcriptional signatures and testing in vivo whether PD cells can generate a PD-like plaque.

Methods. Human TA, PD, and CC cells were grown with transforming growth factor beta 1 (TGFβ1; TA+, PD+, CC+) or without it (TA–, PD–, CC–) and assayed by (i) immunofluorescence, Western blot and RT-PCR for myofibroblast, smooth muscle cell and stem cell markers; (ii) collagen content; and (iii) DNA microarray analysis. The ability of PD+ cells to induce a PD-like plaque in an immuno-suppressed rat model was assessed by Masson trichrome and Picrosirius Red stainings.

Main Outcomes Measures. Fibroproliferative features of PD cells and identification of related key genes as novel targets to reduce plaque size.

Results. Upon TGFβ1stimulation, collagen levels were increased by myofibroblasts in the PD+ but not in the CC+ cells. The transcriptional signature of the PD– cells identified fibroproliferative, myogenic (myofibroblasts), inflammatory, and collagen turnover genes that differentiate them from TA– or CC– cells and respond to TGFβ1 with a PD+ fibrotic phenotype, by upregulation of IGF-1, ACTG2, MYF5, ACTC1, PSTN, COL III, MMP3, and others. The PD+ cells injected into the TA of the rat induce a PD-like plaque.

Conclusions. This suggests a novel combination therapy to eliminate a PD plaque by targeting the identified genes to (i) improve collagenase action by stimulating endogenous metalloproteinases specific to key collagen types and (ii) counteract fibromatosis by inhibiting myofibroblast generation, proliferation, and/or apoptosis. **Gelfand RA, Vernet D, Kovanecz I, Rajfer J, and Gonzalez-Cadavid NF. The transcriptional signatures of cells from the human Peyronie's disease plaque and the ability of these cells to generate a plaque in a rat model suggest potential therapeutic targets. J Sex Med 2015;12:313–327.**

Key Words. Penis; Fibrosis; Fibromatosis; Dupuytren's; Myofibroblast; Fibroblast; Smooth Muscle Cells; Collagenase; Periostin; PSTN; MYF5; IGF-1; NOX4; KTN34; ACTC1; PAI-2; MMP3; ATG2

Introduction

Peyronie's disease (PD) is a relatively prevalent condition affecting about 9% of men. It may present either as a solitary or multiple fibrotic plaques within the tunica albuginea (TA) of the penis which can lead to a penile deformity [1]. This curvature impacts the self-esteem and quality of life of afflicted patients and their partners and is the main reason why most men seek treatment. One option is via surgery, but because of the possibility of penile shortening and/or erectile dysfunction, many men seek a nonsurgical cure.

Current medical therapy has focused on ways to "lessen" or soften the plaque to reduce curvature [2]. The widely used intralesional injections of verapamil or interferon suffer from either a lack of conclusive evidence of its efficacy or severe side effects [1,2]. Recently, the FDA approved intralesional Clostridium histolyticum collagenase to lessen the plaque [3,4]. However, the modest improvement in penile curvature of 16% as well as the fact that only one in five patients obtained a "straight" erection in these recent trials suggests that there is room for improvement. This may be based on basic science investigations reported primarily during the last 15 or so years [5,6].

The PD plaque forms as the result of entrapment of fibrin that extravasates as fibrinogen into the TA following an "injury" to this tissue [7]. The fibrin induces the expression of transforming growth factor β1 (TGFβ1), which initiates the conversion of fibroblasts to myofibroblasts as well as an increase in plasminogen activator inhibitor 1 (PAI-1) that inhibits fibrinolysis, an increase in reactive oxygen species (ROS) that causes oxidative stress and an induction of the inducible nitric oxide synthase (iNOS) [8]. Unknown factors prevent the myofibroblasts from undergoing apoptosis and/or stimulate their continuous formation so that collagen production remains unabated. Therefore, the PD plaque has been defined as a scar or as a wound that never heals [9]. In about 20% of cases, the PD plaque progresses to ossification [10] that results from heterotopic osteogenesis. Successful treatment of a PD plaque in addition to collagenolysis would require treatment modalities, which are able to (i) inhibit collagen production by the myofibroblasts; (ii) induce myofibroblast apoptosis; (iii) inhibit oxidative stress; and/or (iv) prevent or reverse ossification/calcification.

Our laboratory has demonstrated via DNA microarrays, which define global transcriptional signatures, that the PD tissue is in constant turnover, that is, collagen and other extracellular matrix (ECM) production is counteracted by their catabolism [10–13] and by endogenous mechanisms of defense, one of which is the spontaneous induction of iNOS within the TA. iNOS generates a sustained output of nitric oxide (NO) and cGMP that acts as inhibitors of oxidative stress, collagen synthesis, and myofibroblast generation [6,14] and counteract the inflammatory and profibrotic processes that lead to the generation of a plaque. Collagen/ECM catabolism is triggered by the overexpression of certain metalloproteinases (MMPs) [15,16], particularly MMP-2 and MMP-9. However, MMP overexpression may be overcome by either (i) an increase in tissue inhibitors of MMPS (TIMPs) [17]; (ii) by the eventual downregulation of key endogenous MMPs [18]; if catabolism of collagen/ECM [15] prevails over anabolism, the plaque may regress, whereas if the contrary occurs, it continues growing; (iii) the ossification of the PD plaque impairs collagen breakdown and may be counteracted by inhibiting intralesionally the osteogenic differentiation of either myofibroblasts or stem cells, or both [10,14,19].

The interpretation of the transcriptional signatures of the human PD plaque tissue in comparison with those for the nodules of Dupuytren's disease (that is present in 15–20% of PD patients [2]) has provided some useful information but is limited by the heterogeneity of the respective tissues [12,13]. In contrast, the availability of human cell cultures from the PD plaque and non-PD TA, and even of smooth muscle cells (SMCs) derived from the corpora cavernosa (CC) [14,19,20] that can be stimulated by profibrotic effectors should allow for a more focused and controllable comparison of transcriptional gene expression and key phenotypes among these cells. This may allow for the identification of potential therapeutic targets to stimulate collagenolysis and/or inhibit plaque growth, but to our knowledge, no such reports are available. The ability of the myofibroblasts and/or the stem cells present in the human PD cultures to generate a PD-like plaque has not yet been tested. Even if collagen may be broken down by exogenously administered collagenase assisted by other pharmacological treatments, the persistence of proliferating and profibrotic cells in the plaque may defeat the improvement of collagenolysis by inducing plaque regrowth, as shown in Dupuytren's cell cultures [21].

The aim of the current work was to identify novel therapeutic targets for PD treatment by studying the role of human PD cell cultures in