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Sexual Medicine

Alterations in the Transforming Growth Factor (TGF)-β Pathway as a Potential Factor in the Pathogenesis of Peyronie's Disease☆

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Abstract

Objectives: The development of fibrotic diseases is associated with alterations in the transforming growth factor β (TGF-β) pathway. We have investigated the expression and activity of Smad transcription factors of the TGF-β pathway in primary tunical fibroblasts derived from patients with Peyronie's disease and from controls.

Methods: Primary fibroblasts were established from biopsies obtained from plaques of 16 patients with Peyronie's disease or the tunica albuginea of 8 control patients. The expression and activity of Smad transcription factors in control and TGF-β–stimulated primary fibroblasts were investigated at the RNA and protein level by reverse transcription-polymerase chain reaction, Western blotting, and immunofluorescence.

Results: RNA expression levels of Smad3 and Smad4 were significantly increased in fibroblasts from patients with Peyronie's disease. When stimulated with TGF-β1, fibroblasts showed rapid nuclear translocation of Smad2/3, as soon as 15 min after stimulation. This effect was more pronounced and exhibited an earlier onset in fibroblasts from patients with Peyronie's disease, compared with controls. In addition, an increased nuclear retention time of Smad4 was observed in fibroblasts from patients with Peyronie's disease.

Conclusions: The expression and activity of Smad transcription factors of the TGF-β pathway is increased in fibroblasts of patients with Peyronie's disease. Alterations in the TGF-β pathway seem to be a pathogenetic factor in the development of Peyronie's disease.

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1. Introduction

Peyronie's disease is characterized by a fibrotic and sometimes calcified plaque of the tunica albuginea and the adjacent tissue of the corpora cavernosa. The underlying molecular mechanism for the development of this hypertrophic scar tissue remains enigmatic [1]. Repetitive penile microtraumatization during sexual intercourse with subsequent induction of maladaptive wound healing and tissue fibrosis represents one of the main causes of plaque development [2]. In addition, initial fibrin deposition followed by inflammatory reactions has been shown to induce scar formation and plaque development in the tunica albuginea [2].

Recent studies have documented an increased expression of transforming growth factor β1 (TGF-β1) in plaques of patients with Peyronie's disease [3]. This has provided evidence that TGF-β1 plays a crucial role in the pathogenesis of this disease [3]. In addition, genetic variations in the coding region of the TGF-β1 gene have been documented in Peyronie's disease. An increased frequency of homozygosity in the single nucleotide polymorphism (SNP) G915C (G/G) has been detected in patients with Peyronie's disease [4]. This SNP has been associated with elevated TGF-β1 serum levels and the occurrence of fibrotic disorders in other organ systems, such as pulmonary fibrosis [5]. Moreover, intratunical injection of cytomodulin, a synthetic heptapeptide with TGF-β-like activity, led to Peyronie's disease-like conditions in an animal model, further suggesting a causal role for TGF-β in the pathogenesis of this disorder [6].

In recent years, novel aspects of the TGF-β pathway have been elucidated. TGF-β is a soluble and secreted growth factor of the TGF-β superfamily, which includes TGF-βs, activins and bone morphogenetic proteins (BMPs). TGF-β binds to specific serine/threonine kinase receptors at the cell surface, which triggers oligomerization of receptor isotypes, and subsequent phosphorylation and activation of intracellular signalling molecules, the Smad transcription factors. To date, eight different Smad molecules have been cloned and characterized, which have been categorized into three different subgroups: (1) receptor-regulated Smads (R-Smads), (2) common Smads (co-Smads), and (3) inhibitory Smads (I-Smads) [5]. Smad2 and 3, the two R-Smads, have been associated with TGF-β and activin signaling, whereas Smad1, 5 and 8 have been associated with BMP signaling. Phosphorylated R-Smads bind the co-Smad (Smad4), the complex of which then translocates into the nucleus to regulate cellular proliferation and/or differentiation via interaction with regulatory sequences in chromatin [7]. On the basis of these observations, we sought to investigate the expression and function of the TGF-β pathway in primary fibroblasts derived from plaques of patients with Peyronie's disease or from control patients.

2. Patients and methods

2.1. Patients

We obtained biopsies from 16 patients with Peyronie's disease undergoing surgery and 8 control patients. All patients enrolled in this study gave written consent, and the procedures were approved by the internal ethical review board of the University of Giessen School of Medicine. For patients with Peyronie's disease, specimens were obtained directly out of the plaque during surgery, after degloving of the penis using the sleeve technique. In case of a plication procedure ($n = 9$), biopsies were obtained directly out of the plaque that could be easily identified by palpation with the help of a punch biopsy gun (Manan Pro-Mag 1.2, Medical Device Technologies Inc., Gainesville, FL, USA) with a 14-gauge biopsy needle (Manan SACN 14 ga × 8 cm, Medical Device Technologies Inc.). The specimen was directly cut out of the incision line, when an incision and grafting procedure ($n = 7$) was performed. In all patients investigated, an additional specimen was obtained to confirm the clinical diagnosis of Peyronie's disease by histology. In the control group, tunica albuginea was obtained from the incision line during insertion of a penile implant ($n = 6$) or from the ellipsoids excised during a Nesbit procedure ($n = 2$). The diagnosis of a normal tunica albuginea was also confirmed histologically with the use of an additional specimen. Biopsy samples were transferred into sterile vials containing 0.9% NaCl and transferred directly from the operation theatre into the laboratory for the initiation of cell cultures. A cell culture was initiated in all specimens. Fibroblasts grew out in 10 of 16 cases of Peyronie's disease and in 6 of 8 cases of the control group.

2.2. Cell culture

The tissues obtained were cleaned, cut and distributed onto cell culture dishes. Tissue pieces were then submerged in cell culture medium (Cell Growth Medium 2; C-39210; Promocell, Heidelberg, Germany), and fibroblasts were allowed to grow out from the tissues. After fibroblasts had grown out, the tissues were removed and the cells were trypsinized. Cells were further incubated at 37 °C in a humidified 5% CO_2 atmosphere. ISCOVE-Medium basal (Cat# F0465; Biochrom AG, Berlin, Germany) supplemented with 10% fetal calf serum, 1% L-glutamine, and 50 μg/ml gentamicin was used to expand fibroblast cultures. The medium was changed every 2–3 days. Only cells between the 2nd and 12th passages were used throughout this study.

2.3. RNA isolation and reverse transcription-polymerase chain reaction

Total RNA was isolated from fibroblasts with the use of the Rneasy Midi Kit (Quiagen, Hilden, Germany) according to the

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