

COMPARISON OF GENE EXPRESSION PROFILES BETWEEN PEYRONIE'S DISEASE AND DUPUYTREN'S CONTRACTURE

A. QIAN, R. A. MEALS, J. RAJFER, AND N. F. GONZALEZ-CADAVID

ABSTRACT

Objectives. To compare the gene expression alterations in human Peyronie's disease (PD) and Dupuytren's disease (DD) to determine whether they share a common pathophysiology. Multiple mRNA expression profiles of human PD have previously shown that genes that regulate fibroblast replication, myofibroblast differentiation, collagen metabolism, tissue repair, and ossification are involved. DD, a palmar fascia fibrosis, may be associated with PD.

Methods. Total RNA samples from PD plaques, normal tunica albuginea, Dupuytren's nodules, and normal palmar fascia (nine samples per group) were subjected to differential gene expression profile analysis (Clontech Atlas DNA microarray) comparing PD with tunica albuginea and DD with normal palmar fascia. Changes of more than 2.0 in PD and DD compared with tunica albuginea and normal palmar fascia, respectively, were recorded. Reverse transcriptase-polymerase chain reactions were performed for some genes whose expression was altered in PD.

Results. Some of the gene families upregulated in both PD and DD were (a) collagen degradation: matrix metalloproteinase (MMP), with MMP2 and MMP9, and thymosins (MMP activators), with TM β 10 and TM β 4; (b) ossification: osteoblast-specific factors (OSFs) OSF-1 and OSF-2 (DD only); and (c) myofibroblast differentiation: RhoGDP dissociation inhibitor 1. The genes upregulated in PD only were decorin (an inhibitor of transforming growth factor- β 1 and a part of fibroblast replication/collagen synthesis) and early growth response protein. Reverse transcriptase-polymerase chain reaction confirmed these changes.

Conclusions. These data demonstrate that the pattern of alterations in the expression of certain gene families in PD and DD is similar, suggesting that they share a common pathophysiology and may be amenable to the same therapeutic regimens. UROLOGY 64: 399-404, 2004. © 2004 Elsevier Inc.

Peyronie's disease (PD) is a localized fibromatosis in the tunica albuginea (TA) of the penis, leading to penile deformation, often associated with erectile dysfunction and/or pain.¹ It is assumed to be caused by trauma to the erect penis, resulting in extravasation of fibrin and other blood proteins into the TA that, together with other unknown factors, elicit an inflammatory reaction followed by the production of pro-fibrotic agents,

such as transforming growth factor- β 1 (TGF- β 1) and reactive oxygen species.^{2,3} These pro-fibrotic factors promote collagen synthesis and/or inhibit its breakdown and stimulate the differentiation of fibroblasts in the TA into myofibroblasts,⁴⁻⁶ cells that share the phenotype of fibroblasts and smooth muscle and are critical factors in wound healing.

Myofibroblasts normally disappear by apoptosis after wound healing, but they can persist abnormally in incomplete wound healing and accumulate in fibrotic processes.⁷ Primary cultures of fibroblasts differentiating into myofibroblasts within the PD plaque and normal TA have been characterized.^{5,6,8} To counteract fibrosis, these fibroblasts and myofibroblasts express inducible nitric oxide synthase, which produces a continuous output of nitric oxide that has been proposed to inhibit TGF- β 1 synthesis, quench reactive oxygen species, reduce myofibroblast differentiation, and

These studies were funded mainly by a grant from the Eli and Edythe L. Broad Foundation and in part by National Institute of Health grants R01DK-53069 and G12RR-03026.

From the Harbor-UCLA Research and Education Institute, Torrance, California; and Departments of Orthopedics and Urology, University of California, Los Angeles, School of Medicine, Los Angeles, California

Reprint requests: Nestor F. Gonzalez-Cadavid, Ph.D., Department of Urology, Harbor-UCLA Research and Education Institute, Building F-6, 1124 West Carson Street, Torrance, CA 90502

Submitted: December 29, 2003, accepted (with revisions): April 7, 2004

promote collagen breakdown by activating metalloproteinases (MMPs).⁴⁻⁸

PD is often accompanied (21% in one report) by another fibrosis, Dupuytren's disease (DD), occurring in the palmar fascia and characterized by similar fibrotic alterations, although its relationship to trauma is less established.⁹⁻¹¹ As in PD, the myofibroblast is an essential cellular component of the DD nodules.¹¹ However, it is not known whether, irrespective of etiology, PD and DD share a common pathophysiology and a similar interplay of pro-fibrotic and antifibrotic mechanisms.

The advent of the DNA microarrays for the differential analysis of expression of multiple genes¹² allowed us to establish the proof of concept in PD by defining gene expression variations between PD and TA in a small number of patients.¹³ In the present work, we have extended these observations to a larger number of specimens and compared the results with those obtained in DD nodules versus normal tendon tissue and in fibroblast/myofibroblast cell cultures from PD versus the homologous cells cultured from normal TA.

MATERIAL AND METHODS

TISSUES AND CELL CULTURES

PD plaques were excised from the penis of 9 patients who underwent tunica incision/excision and/or venous grafting or insertion of a penile prosthesis. The control TA tissue was obtained from 2 patients undergoing penectomy to treat penile cancer (tissue distant from the tumor) and 7 patients with erectile dysfunction undergoing penile prosthesis surgery who did not have PD. The mean patient age for those with PD was 58 years (range 54 to 62) and for those with normal TA was 59 years (range 43 to 69). For the samples of DD, the involved palmar fascia was obtained at fasciectomy from 9 patients, and the control tissue was obtained from adjacent noncompromised tendon (mean age 68 years, range 58 to 76). All subjects provided written informed consent under institutional review board approval. Total RNA was isolated from 50 to 100 mg of tissue, except for the DD control ligament (less than 20 mg), using the TriZol procedure and was quality verified by analytical gel electrophoresis.¹³

Fibroblast/myofibroblast cell cultures were obtained from one PD plaque and one normal TA tissue samples,^{5,8} using for analysis representative samples at passages 7 and 9. Immunostaining for vimentin established the purity of the culture, and alpha-smooth muscle actin detected myofibroblasts.^{5,8} The RNA was isolated as above.

DNA MICROARRAY ASSAY

Total RNA samples from each of the individual specimens were digested with RNase-free DNase I and reverse transcribed. cDNA samples were labeled with phosphorus-32[deoxyadenosine triphosphate] using a primer mix for 1176 genes in the Human Atlas 1.2 array kit (Clontech, Palo Alto, Calif).¹³ The labeled cDNA samples were separately hybridized (total 40) against a pair of identical nylon membranes for which each cDNA was represented as a single spot distributed in panels of functionally related genes. The radioactive signals were visualized by both autoradiography and phosphor imaging, and a comparison of the intensities of each signal was performed between the following RNA samples: PD plaque

versus normal TA; DD nodule versus control ligament; and PD cells versus TA cells (Atlas Image 1.0; 20 paired comparisons total). Values were corrected for differences in hybridization efficiency between the two membranes by dividing the average expression of all genes in the respective arrays ("global normalization"). Adjusted values with relative differences in gene expression of more than 2.0 up or down were selected.

REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION CONFIRMATION

RNA was reverse transcribed using random priming¹³ followed by semiquantitative polymerase chain reaction (PCR) analysis. The 5' ends of the forward and reverse primers corresponded, respectively, to the following nucleotide positions on the human gene sequences in GenBank: pleiotrophin (osteoblast-specific factor [OSF]-1; 39/420/accension number [AN] X52946); MMP2 (477/778/AN J03210); decorin (686/922/AN M14219); thymosin β (TMSB)-10 (40/342/AN M92381); TMSB-4 (300/496/AN M17733); and glyceraldehyde phosphate dehydrogenase (75/1083/AN XM006959). The reactions were conducted with glyceraldehyde phosphate dehydrogenase as the reference gene for 25 cycles. DNA fragments were separated by electrophoresis, visualized by ethidium bromide, and scanned. Band intensities were determined by densitometry and normalized to the respective glyceraldehyde phosphate dehydrogenase band.

STATISTICAL ANALYSIS

Values are expressed as the mean \pm standard error or as the range. For DNA microarrays, these values were obtained from the computer analysis program as ratios; therefore, no statistical treatment was applied. For reverse transcriptase (RT)-PCR, the normality distribution of the data was established using the Wilk-Shapiro test, and the outcome measures between the PD and control groups were compared by the two-tier *t* test. Differences were considered statistically significant at $P < 0.05$ (Graph Pad Prism, version 3.0, program).

RESULTS

A series of 15 genes were upregulated and none were downregulated in the PD plaque versus the normal TA in at least 2 of the 9 patients. In the DD nodules versus the control ligament, 16 and 3 genes were upregulated and downregulated, respectively, in at least 4 of the patients. In the fibroblasts cultured from the PD plaque compared with the ones from the normal TA, 10 genes were upregulated and none were downregulated.

Table I shows that eight genes relevant to fibrosis were upregulated in both PD plaques and DD, and six of these same genes were also upregulated in the PD fibroblast cultures. None were downregulated. Also, eight genes were upregulated only in DD, five only in the PD plaques, and one was downregulated only in DD; with two genes affected in opposite directions in the PD plaques and DD. In the PD plaques, changes agreeing with the variation represented by the mean value occurred for most genes in less than one half of the patients. In contrast, in the DD nodules, the upregulation in gene expression depicted by the mean value occurred for five genes in 7 or more of 9 patients. In

TABLE 1. Alterations of gene expression in Dupuytren's nodules, Peyronie's plaques, and cultured fibroblasts, determined with DNA microarray assay

Protein/Gene	Dupuytren's Nodules		Peyronie's Plaques		Peyronie's Fibroblasts	
	n	Mean ± SE	n	Mean ± SE	n	Mean ± Range or Individual Value
Matrix metalloproteinase 2 (MMP-2); 72-kDa gelatinase A	9	29 ± 10	2	4.7 ± 2.6		
Matrix metalloproteinase 9 (MMP-9)			2	50.8 ± 0.8		
Thymosin beta-10 (TMSB-10)	9	5.9 ± 2.6	5	5.5 ± 1.3	2	2 ± 0.26
Thymosin beta-4; FX	8	5.9 ± 1.5	5	2.5 ± 0.9	2	1.9 ± 0.1
Cortactin; amplexin; ems-1 oncogene	8	22.6 ± 7.9	4	15.5 ± 12.2	2	4.8 ± 2.9
Transforming protein RhoA H12 (Rho12)	6	2.6 ± 0.5	3	5.5 ± 2.3	1	1.7
RhoGDP dissociation inhibitor 1 (Rho-GDI 1); Rho-GDI alpha (GDIA1); ARHGDI	6	3.5 ± 1.4	2	18.3 ± 2.4	1	4.8
Pleiotrophin precursor (PTN); osteoblast specific factor 1 (OSF-1)	5	5.6 ± 1.4	3	4.3 ± 0.5		
Osteoblast specific factor 2 (OSF-2); OSF-2P1	4	26.7 ± 12.7				
Amyloid A4 protein precursor; nexin II (PN-II)	5	5.8 ± 2.0	3	2.4 ± 0.8	1	2.4
Defender against cell death 1 (DAD-1)	7	4.0 ± 0.5			1	3.3
Ubiquitin	6	3.4 ± 0.3				
Heat shock 27-kDa protein (HSP27)	5	2.9 ± 1.1			1	2.8
Procollagen alpha 2 subunit	6	10.4 ± 5.2				
Macrophage-specific stimulating factor (MCSF or CSF1)	5	6.4 ± 3.7			1	2.4
Guanine nucleotide-binding protein Gs alpha subunit	6	5.1 ± 2.6				
Alpha 1 catenin; cadherin-associated protein	5	2.7 ± 1.5				
Transcription factor AP-1			6	5.8 ± 1.1		
Early growth response protein 1 (hEGR1)			6	6.2 ± 1.2		
Monocyte chemotactic protein 1 (MCP1)			4	9.2 ± 5.4		
Bone proteoglycan II precursor (PGS2); decorin (DCN)	6	0.5 ± 0.1	5	2.5 ± 0.3		
T-cell-specific rantes protein precursor	4	0.3 ± 0.1	3	2.5 ± 0.8		
Integrin beta-1			4	3.2 ± 0.6	2	6.1 ± 4.4
Copper-transporting ATPase 2; copper pump 2	4	0.1 ± 0				

Matched comparisons between individual diseased (n = 9) and control (n = 9) tissues from each patient chosen at random, were performed within Peyronie's and Dupuytren's groups; comparisons for Peyronie's cells involved an n = 2, making a total of 20 individual matched hybridizations in this study; n represents number of comparisons exhibiting a more than twofold change in same direction as mean value.

the case of the PD fibroblast cultures, seven and nine genes showed the same alterations as in the PD and DD tissues, respectively.

Of the genes upregulated in both PD and DD, the ones most prominently increased were MMPs involved in collagen breakdown,¹⁴ specifically MMP-2 in all the DD nodules and either MMP-2 or MMP-9 in one half of the PD plaques. This was not reflected in the PD fibroblast cultures. The second group of overexpressed genes were the thymosins, peptides that are activators of MMPs and that interact with fibrin.^{15,16} TMSB-10 and TMSB-4 were upregulated in nine and eight, respectively, of nine DD nodules, and one or the other thymosins was

upregulated in five PD plaques. Both genes were also overexpressed in the PD fibroblast cultures.

Cortactin or amplexin, a gene involved in actin/cytoskeleton interactions required for fibroblasts and myofibroblasts to generate the contractile forces that are operative in wound healing,¹⁷ was overexpressed in most of the DD nodules but in only one half of the PD plaques. PD fibroblast cultures also overexpressed this gene. This pattern was also seen in DD, PD plaques, and PD fibroblast cultures with other genes with a similar function and additional involvement in controlling apoptosis (eg, RhoGDP dissociation inhibitor 1 and transforming protein RhoA H12).¹⁸ Either OSF-1 or

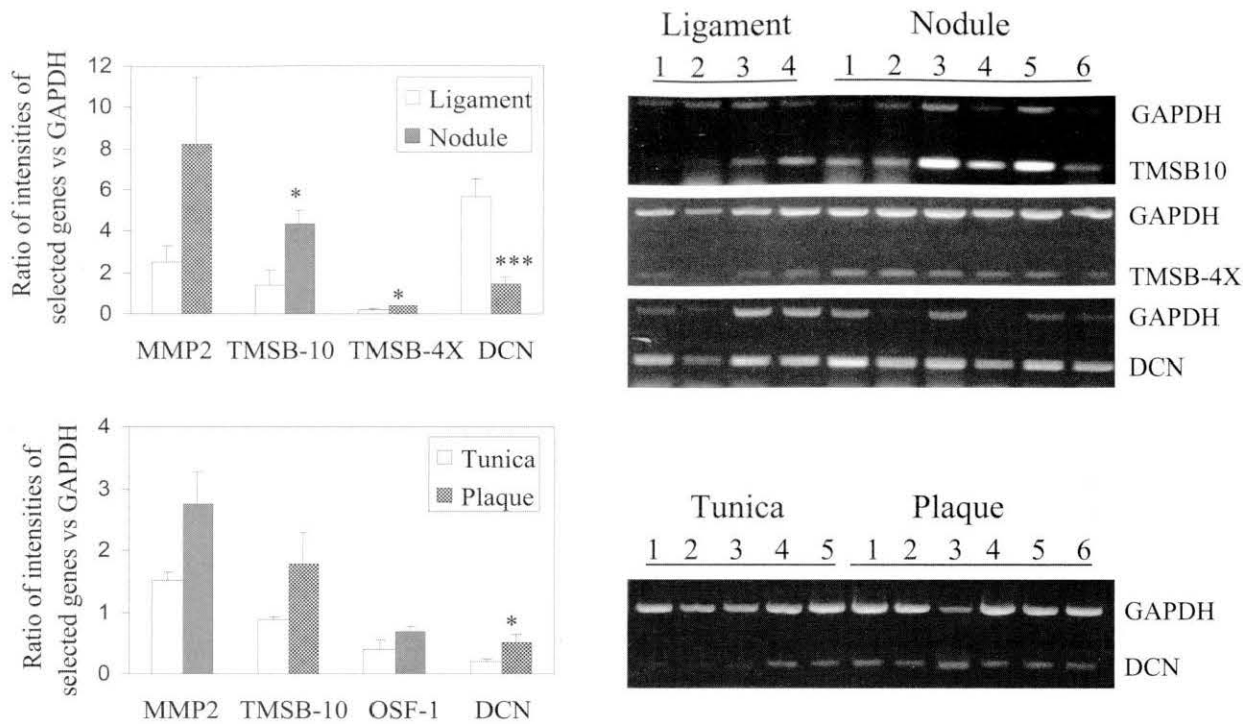


FIGURE 1. Confirmation by RT-PCR of alterations in expression of some selected genes in PD plaques and DD nodules. Total RNA samples ($n = 4$ to 6) were submitted to RT-PCR for genes specified compared with RNA samples from respective control tissues. (Right) DNA bands stained with ethidium bromide on agarose gels for selected genes. (Left) Band intensities evaluated by densitometry and corrected by value for glyceraldehyde phosphate dehydrogenase reference gene. * $P < 0.05$; *** $P < 0.001$.

OSF-2^{19,20} was overexpressed in every DD nodule; in the PD plaque, only OSF-1 was overexpressed, but only in 1 of 3 of patients, with no alterations in OSF-2 levels. Neither of these two genes were overexpressed in the PD fibroblast cultures. A protease inhibitor, amyloid A4 protein,^{21,22} associated with inflammation and apoptosis, was also stimulated in less than one half of the patients in both DD and PD plaques.

Differential alterations in expression between DD and PD plaques were observed with four proteins also associated with apoptosis, proteolysis, and inflammation: defender against cell death-1,²³ ubiquitin,²⁴ heat shock 27-kDa protein,¹³ and macrophage-specific stimulatory factor,²⁵ as well as the most obvious indicator of fibrosis, the collagen subunit procollagen alpha2. These mRNAs were upregulated in most DD nodules, but not in PD plaques, although they (except for collagen and ubiquitin) were modestly increased in the PD fibroblast cultures. In contrast, the general transcription factor AP1²⁶ and immediate early growth response gene (hEGRI), both associated with fibroblast replication, and monocyte chemoattractant protein-1, involved in inflammation and detected in our earlier report,¹³ were upregulated in several PD plaques, but not in DD or the PD cells. Two genes exhibited the opposite behavior in the

PD plaques and DD: decorin, an anti-TGF- β 1 factor,²⁷ and T-cell-specific rantes protein precursor, associated with inflammation and monocyte chemoattractant protein-1 induction,²⁸ both of which were reduced in DD and upregulated in PD, but not changed in the PD cells.

Confirmation of these alterations was obtained in some selected cases by semiquantitative RT-PCR (Fig. 1). MMP2 was, as expected from the microarrays, upregulated in both DD and PD, albeit to a much lower extent, probably reflecting the lower sensitivity and higher variability of RT-PCR. A similar situation was found with TMSB-10, and, in the case of DD (not assayed in PD), with TMSB-4. OSF-1 was assayed in PD and not in DD and showed a more modest upregulation than in the microarrays. Finally, decorin showed the behavior detected with microarrays (ie, downregulation in DD versus an increase in PD).

COMMENT

These data extend and confirm our preliminary results demonstrating the usefulness of the DNA microarrays to define changes in mRNA levels in PD in relation to normal TA^{1,13} and establish the first comparison of multiple gene expression in two fibrotic conditions, PD and DD, which are fre-

quently associated. In addition, this method identified genes that undergo changes of expression in the PD fibroblast cultures similar to those found in the original PD tissue. Three main features were observed: (a) the heterogeneity of PD plaques compared with DD nodules in terms of pathophysiology, as revealed by the lower number of patients with PD displaying a common dysregulation of expression for any given gene, in agreement with the different progression of both diseases^{1,9}; (b) the existence of some genes with similar alterations in mRNA levels compared with their normal tissues in both diseases, maintained in most cases in the PD cells, particularly for MMPs, thymosins, OSFs, and genes involved in fibroblast apoptosis and differentiation, suggesting tissue remodeling; and (c) individual genes also belonging to apoptotic and inflammation-related pathways whose expression is affected specifically in PD compared with DD.

The most remarkable changes in gene expression were the ones related to defense mechanisms against fibrosis, which confirmed that the PD plaque and DD nodules are in active remodeling and not in terminal involution.^{1,13} Thymosins in both tissues, as well as in the PD fibroblasts, and decorin only in PD, were overexpressed, probably trying to overcome and/or inhibit inflammation, defective collagen breakdown, and the pro-fibrotic effector TGF- β . Thymosins are the major G-actin sequestering proteins: TMSB-4 can accelerate healing of skin wounds and burns in the cornea, and TMSB-10 is pro-apoptotic and anti-inflammatory.^{15,16} Both thymosins induce MMP, whose high levels in both PD and DD may represent an unsuccessful attempt to reduce collagen deposition, owing to resistance by either cross-links caused by advanced glycation end products²⁹ or by inhibition of MMP activity through accumulation of MMP inhibitors.¹⁴ In PD, the impaired collagen breakdown may become, at the late stage of fibrosis found in most surgically excised plaques, more deleterious than excessive collagen synthesis, because no increase in collagen mRNA was detected, in contrast to the findings in our previous study.¹³

The remodeling caused by myofibroblast differentiation and apoptosis³⁰ is also apparent in the upregulation of pro-apoptotic Rho proteins, cortactin, and amyloid protein precursor in both tissues and the PD cells and only in DD and PD cells for pro-apoptotic ubiquitin and heat shock 27-kDa protein and antiapoptotic defender against cell death-1. The Rho family is of particular note because RhoA H12 is an intracellular small GTPase¹⁸ involved in apoptosis, contractility (cytoskeleton), myofibroblast differentiation, and blockade of growth and regeneration. The inhibitor Rho-GDI 1 keeps Rho proteins in the inactive stage, its overexpression blocks the myofibroblast phenotype

and collagen synthesis, and its cleavage by caspase-3 stimulates apoptosis, so that Rho-GDI 1 is likely to act as an antifibrotic factor, antagonizing Rho proteins. Interestingly, RhoA/Rho kinase signaling counteracts penile erection and is inhibited by nitric oxide.³¹

Cortactin¹⁷ is also a target for caspase 3 during apoptosis and a key regulator of actin cytoskeleton remodeling, being the effector of fibroblast growth factor-1 signaling for cell migration and interaction with the extracellular matrix. An interesting finding was the upregulation of the Alzheimer protein, amyloid A4 protein precursor,^{21,22} seen in 60% and 33% of the patients with DD and PD, respectively, accompanied by a significant reduction in copper-transporting adenosine triphosphatase 2 in every one of the DD nodules in which amyloid A4 protein precursor was increased. This is in agreement with amyloid A4 protein precursor binding copper and being the major regulator of copper homeostasis,³² which in turn is involved in inflammation, oxidative stress, and reactive oxygen species production, a major putative cause of fibrosis in general and in PD in particular.¹⁻⁸

Another pathologically meaningful alteration is the upregulation of OSF-1 and/or OSF-2 in PD, in which the plaque is frequently associated with ossification. Surprisingly, however, it was also seen in DD, in which ossification does not appear to take place. We have found pluripotent cells in fibroblast/myofibroblast cultures from the PD plaque that were able to undergo osteogenesis and calcification (unpublished data), and these cells may be present in DD, as judged by the OSF-1 and/or OSF-2 overexpression in every nodule.

CONCLUSIONS

By looking at the endogenous pattern of gene expression, this study provides targets of potential pharmacologic modulation of the levels of genes associated with antifibrotic mechanisms. An obvious strategy is the upregulation of TMSB and decorin and, alternatively, the activation of MMPs by downregulation of their inhibitors. The stimulation of myofibroblast apoptosis and blockade of its differentiation with Rho inhibitors or cortactin may be also beneficial, because accumulation of these cells in an abnormal healing process subsequent to trauma may relate to the fibrosis seen in PD and DD.⁵⁻⁸ Treatment with L-arginine and phosphodiesterase inhibitors, both stimulating apoptosis and remodeling by nitric oxide/cyclic guanosine monophosphate or cyclic guanosine monophosphate alone, respectively, has been shown to prevent the fibrotic plaque in the TGF- β animal model of PD.⁸ We are now determining

whether these alterations in mRNA expression translate into their respective protein levels.

ACKNOWLEDGMENT. To Dr. Martin Gelbard for kindly providing PD specimens.

REFERENCES

1. Gholami SS, Gonzalez-Cadavid NF, Lin C-S, *et al*: Peyronie's disease: a review. *J Urol* **169**: 1234–1241, 2002.
2. Somers KD, and Dawson DM: Fibrin deposition in Peyronie's disease plaque. *J Urol* **157**: 311–315, 1997.
3. Davila H, Ferrini M, Rajfer J, *et al*: Fibrin induction of a Peyronie's-like plaque in the rat penile tunica albuginea: a new model for Peyronie's disease. *Br J Urol* **91**: 830–838, 2003.
4. Ferrini MG, Vernet D, Magee TR, *et al*: Antifibrotic role of inducible nitric oxide synthase (iNOS). *Nitric Oxide* **6**: 1–12, 2002.
5. Vernet D, Ferrini MG, Valente E, *et al*: Effect of nitric oxide on fibroblast differentiation into myofibroblasts in cell cultures from the Peyronie's fibrotic plaque and in its rat model in vivo. *Nitric Oxide* **7**: 262–276, 2002.
6. Mulhall JP: Expanding the paradigm for plaque development in Peyronie's disease. *Int J Impot Res* **15**: S93–S102, 2003.
7. Powell DW, Mifflin RC, Valentich JD, *et al*: Myofibroblasts. I. Paracrine cells important in health and disease. *Am J Physiol* **277**: C1–C9, 1999.
8. Valente EG, Ferrini MG, Vernet D, *et al*: PDE L-arginine and PDE inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. *Nitric Oxide* **9**: 229–244, 2003.
9. Fitzgerald AM, Kirkpatrick JJ, and Naylor IL: Dupuytren's disease: the way forward? *J Hand Surg [Br]* **24**: 395–399, 1999.
10. Carrieri MP, Serraino D, Palmiotto F, *et al*: A case-control study on risk factors for Peyronie's disease. *J Clin Epidemiol* **51**: 511–515, 1998.
11. Tomasek JJ, Vaughan MB, and Haaksma CJ: Cellular structure and biology of Dupuytren's disease. *Hand Clin* **15**: 21–34, 1999.
12. Forster T, Roy D, and Ghazal P: Experiments using microarray technology: limitations and standard operating procedures. *J Endocrinol* **178**: 195–204, 2003.
13. Magee TR, Qian A, Rajfer J, *et al*: Gene expression profiles in the Peyronie's disease plaque. *Urology* **59**: 451–457, 2002.
14. Ravanti L, and Kahari VM: Matrix metalloproteinases in wound repair. *Int J Mol Med* **6**: 391–407, 2000.
15. Huff T, Muller CS, Otto AM, *et al*: Beta-thymosins, small acidic peptides with multiple functions. *Int J Biochem Cell Biol* **33**: 205–220, 2001.
16. Philp D, Badamchian M, Scheremeta B, *et al*: Thymosin beta 4 and a synthetic peptide containing its actin-binding domain promote dermal wound repair in db/db diabetic mice and in aged mice. *Wound Repair Regen* **11**: 19–24, 2003.
17. May RC: The Arp2/3 complex: a central regulator of the actin cytoskeleton. *Cell Mol Life Sci* **58**: 1607–1626, 2001.
18. Olofsson B: Rho guanine dissociation inhibitors: pivotal molecules in cellular signaling. *Cell Signal* **11**: 545–554, 1999.
19. Tare RS, Oreffo RO, Clarke NM, *et al*: Pleiotrophin/osteoblast-stimulating factor 1: dissecting its diverse functions in bone formation. *J Bone Miner Res* **17**: 2009–2020, 2002.
20. Gordeladze JO, Drevon CA, Syversen U, *et al*: Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: impact on differentiation markers, apoptosis, and osteoclastic signaling. *J Cell Biochem* **85**: 825–836, 2002.
21. Hashimoto Y, Niikura T, Chiba T, *et al*: The cytoplasmic domain of Alzheimer's amyloid-beta protein precursor causes sustained apoptosis signal-regulating kinase 1/c-Jun NH2-terminal kinase-mediated neurotoxic signal via dimerization. *J Pharmacol Exp Ther* **306**: 889–902, 2003.
22. Nishimura I, Takazaki R, Kuwako K, *et al*: Upregulation and antiapoptotic role of endogenous Alzheimer amyloid precursor protein in dorsal root ganglion neurons. *Exp Cell Res* **286**: 241–251, 2003.
23. Teruyama K, Abe M, Nakano T, *et al*: Role of transcription factor Ets-1 in the apoptosis of human vascular endothelial cells. *J Cell Physiol* **188**: 243–252, 2001.
24. Lee JC, and Peter ME: Regulation of apoptosis by ubiquitination. *Immunol Rev* **193**: 39–47, 2003.
25. Lenda DM, Kikawada E, Stanley ER, *et al*: Reduced macrophage recruitment, proliferation, and activation in colony-stimulating factor-1-deficient mice results in decreased tubular apoptosis during renal inflammation. *J Immunol* **170**: 3254–3262, 2003.
26. Angel P, and Szabowski A: Function of AP-1 target genes in mesenchymal-epithelial cross-talk in skin. *Biochem Pharmacol* **64**: 949–956, 2002.
27. Reed CC, and Iozzo RV: The role of decorin in collagen fibrillogenesis and skin homeostasis. *Glycoconj J* **19**: 249–255, 2002.
28. Kaufmann A, Salentin R, Meyer RG, *et al*: Defense against influenza A virus infection: essential role of the chemokine system. *Immunobiology* **204**: 603–613, 2001.
29. Forbes JM, Thallas V, Thomas MC, *et al*: The breakdown of preexisting advanced glycation end products is associated with reduced renal fibrosis in experimental diabetes. *FASEB J* **17**: 1762–1764, 2003.
30. Gabbiani G: The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol* **200**: 500–503, 2003.
31. Mills TM, Chitale K, Lewis RW, *et al*: Nitric oxide inhibits RhoA/Rho-kinase signaling to cause penile erection. *Eur J Pharmacol* **439**: 173–174, 2002.
32. Barnham KJ, McKinstry WJ, Multhaup G, *et al*: Structure of the Alzheimer's disease amyloid precursor protein copper binding domain: a regulator of neuronal copper homeostasis. *J Biol Chem* **278**: 17401–17407, 2003.