TGF ß, Collagen-Keloid and the Abnormal Mature Collagen Hypothesis

A different view of fibroids

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Outline:

1. Does TGFβ play a role in development?

2. Describe ECM and mature collagen.

3. Are Fibroids similar to Keloids?

4. Why treat fibroids with pirfenidone?

Leiomyoma:

- Clonal growths
- Arise from a single uterine cell
- Growth promoted by sex steroids
- Cytogenetic studies
- Genetic syndromes
- Few model systems

Myers ER, Obstet. Gynecol., July 2002

Leiomyoma Questions:

– **Why so common?**

– **If a tumor, why almost never cancerous?**

– **Why the different locations?**

– **Do genetic syndromes provide insight?**

– **Why the racial-ethnic differences in disease?**

Cumulative Incidence of Hysterectomy, by race
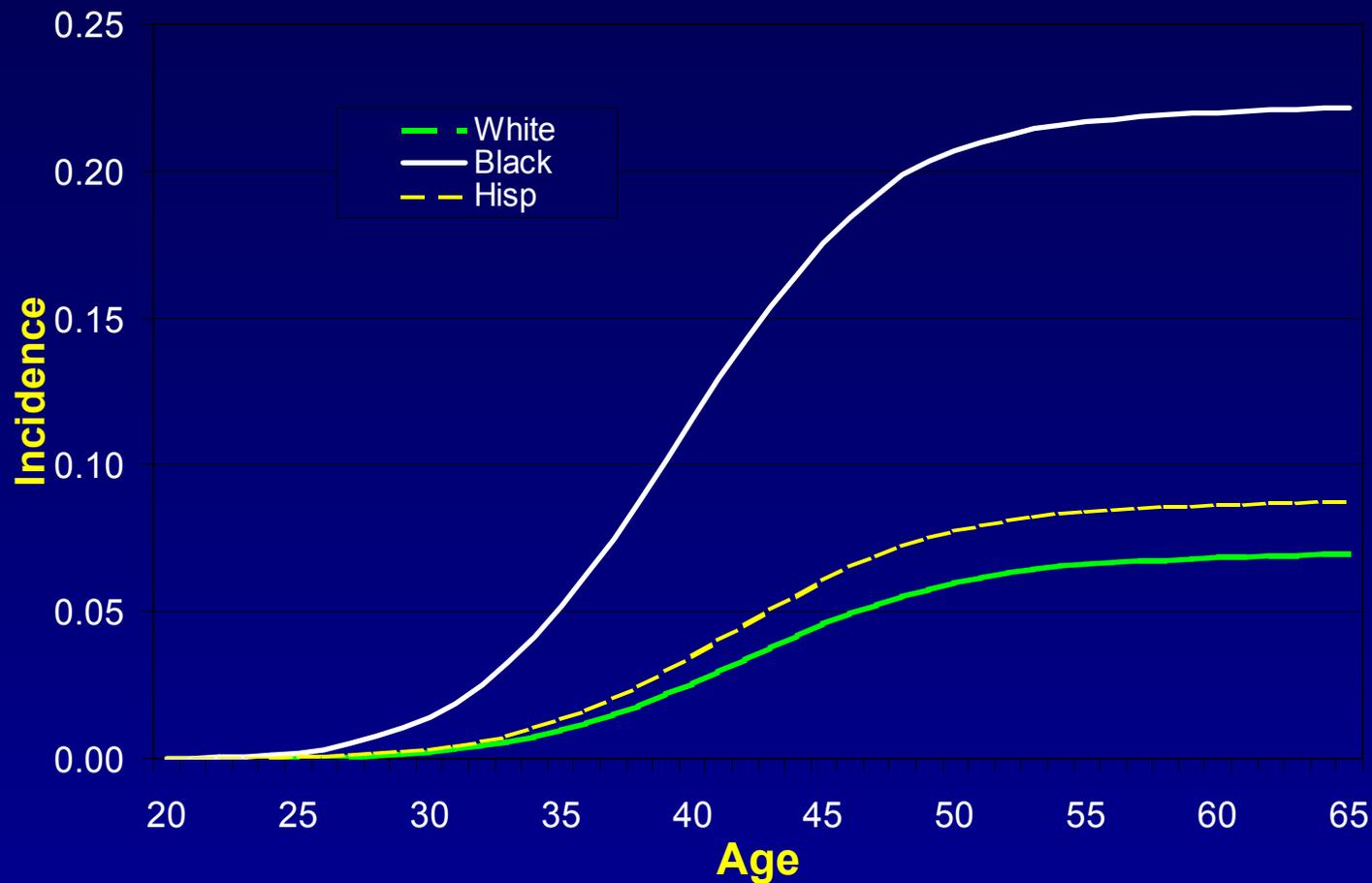
*From E. Myers White House lecture, June, 2002*

Approach:

Global expression profiling of leiomyoma cells "myofibroblasts"

- **Genome-wide screening**

- **Well-suited to comparison: clonal tumors**

- **Use to develop more complex hypotheses**

Tsibris J, et al. Fertil. Steril., July, 2002
Catherino, W, et al. Fertil. Steril., August, 2003

What is a cDNA microarray?
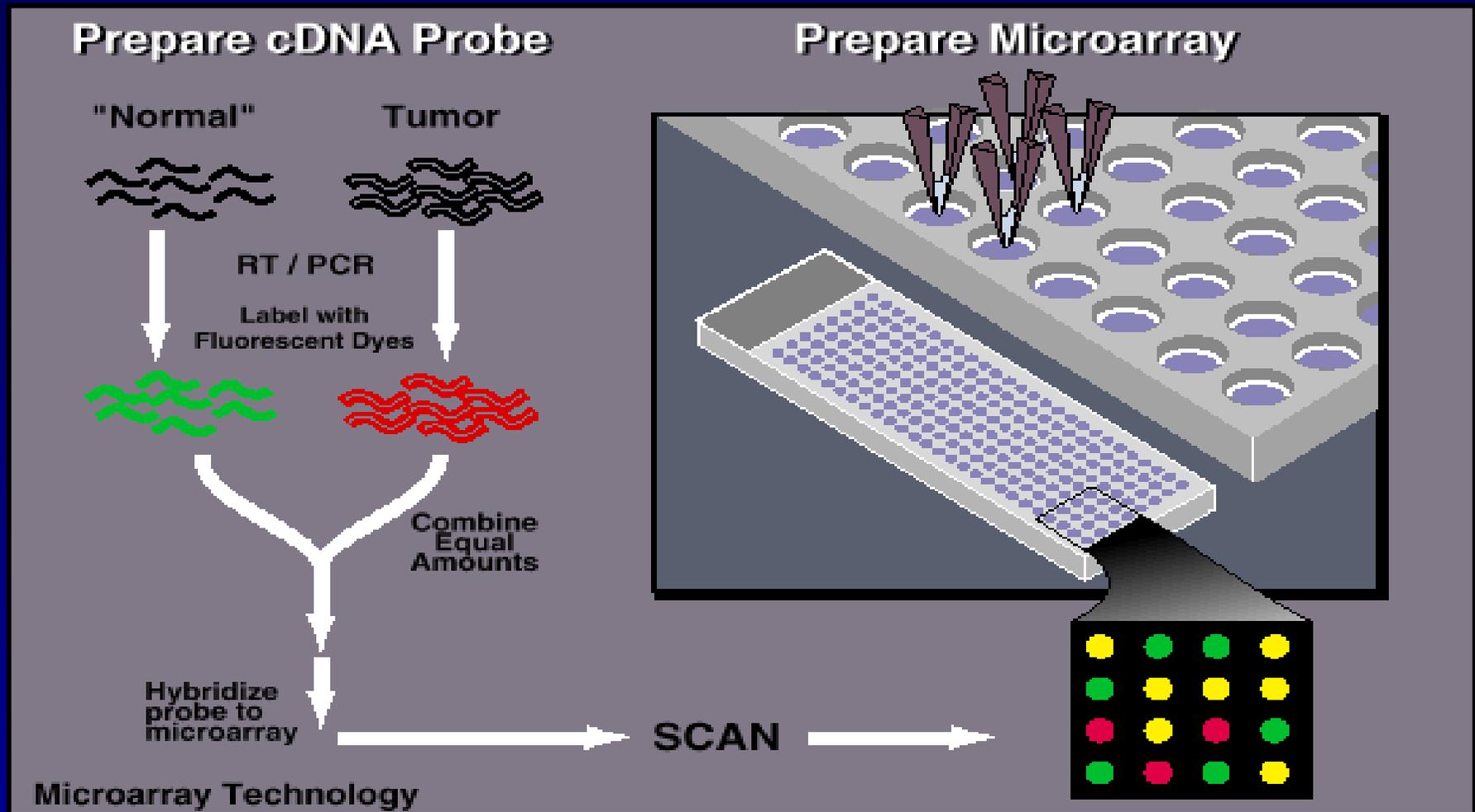
- **Samples from 5 hysterectomies**

- **15 μg total RNA, dscDNA, Biotin-cRNA**

- *Affymetrix* **U133A/B: 30,000 genes**

- **Hewlett-Packard GeneArray Scanner**

- **GeneChip software**

- **>2.0 fold, pixel intensity cutoff 250U**

- **control=normal myometrium**

Microarray results were not identical between laboratories

Gene	U95A fold change Fibroid / Myometrium	U133A fold change Fibroid / Myometrium
Dlk	70.01	0.62 (-1.62)
Frizzled-2	8.59	1.78
CD24ST	8.48	14.98

Sex Steroid-Associated Genes

	F/M (low-high)
• Estrogen receptor α	0.75 (-1.33)
• Estrogen receptor β	0.71 (-1.41)
• Progesterone Receptor	0.98 (-1.02)
• CREBBP	0.74 (-1.35)
• Growth Hormone Receptor	0.83 (-1.21)
• Prolactin Receptor	1.02
• SRC-1	1.47
• P300-CBP	1.52

Extracellular-Matrix Genes

		F/M (low-high)
•	Caldesmon	0.19 (-5.21)
•	Dermatopontin	0.23 (-4.26)
•	Tight Junction Protein-1	0.36 (-2.78)
•	Decorin, Variant C	0.44 (-2.25)
•	Desmin	0.78 (-1.29)
•	Decorin, Variant A	0.85 (-1.17)
•	Decorin, Variant B	1.43
•	P311	3.68
•	Versican	5.79

TGFβ: promoter of connective tissue role in fibrosis, oncogenesis

	Fold F/M
Transforming Growth Factor β3	3.0
Transforming Growth Factor β1	0.71 (-1.4)
Decorin, Variant A	0.85 (-1.17)
Decorin, Variant B	1.43
Decorin, Variant C	0.44 (-2.25)
Interleukin-4 Receptor	0.92 (-1.09)

Reverse Transcriptase-PCR
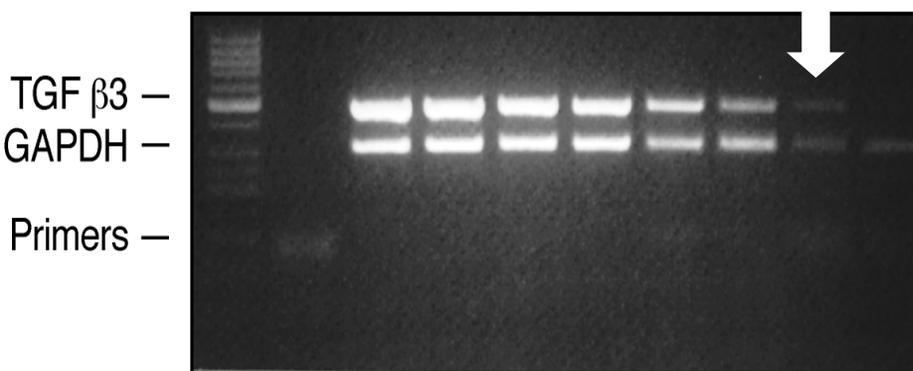
Decorin —
GAPDH —

Primers —

TGF β1 —
GAPDH —

Primers —

TGF β3 —
GAPDH —

Primers —

IL-4 —
GAPDH —

Primers —

Marker | Neg Control | Fibroid | Myometrium | Fibroid | Myometrium | Fibroid | Myometrium | Fibroid | Myometrium

10 ng | 1 ng | 100 pg | 10 pg
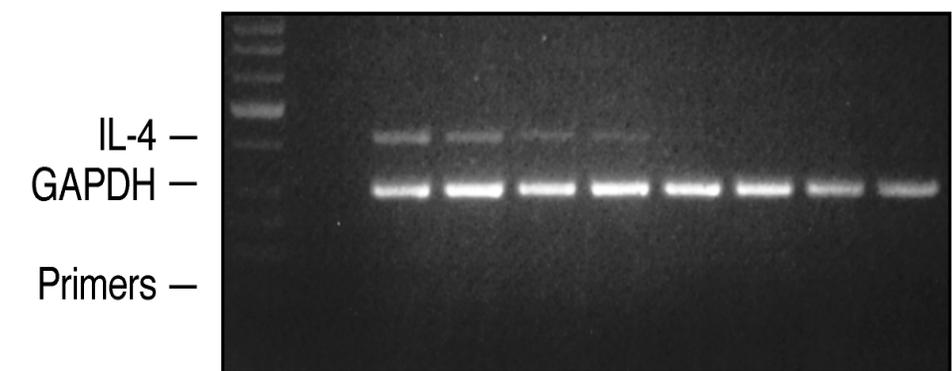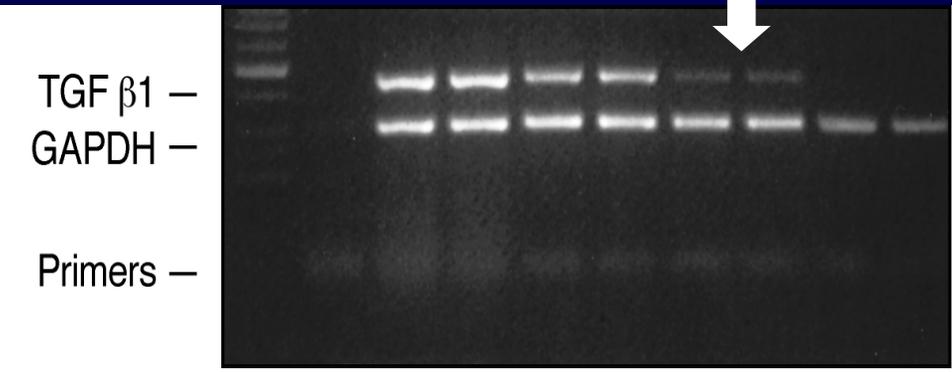
Marker | Neg Control | Fibroid | Myometrium | Fibroid | Myometrium | Fibroid | Myometrium | Fibroid | Myometrium

1 μg | 100 ng | 10 ng | 1 ng

Catherino, et al. Genes, Chromosomes & Cancer 2004.

Collagen Genes in Leiomyoma

Up-regulated	F/M	Down-regulated	F/M
Type I a1	2.61	Type I a1	-1.60
Type IV a2	2.21	Type IV a4	-7.44
Type IV a5	2.19	Type VIII a2	-9.60
Type V a1	2.14	Type XIV a1	-2.17
Type VII a1	3.83		
Type IX a2	2.25		
Type XVIII a1	2.87		

Weigerts iron hematoxylin Beibrich Scarlet-acid Fuschin (40X)

Picrosirus Red stain (20X)

41,000X

Catherino, et al. Genes, Chromosomes & Cancer, 2004.

Leiomyomata   Myometrium

Light Microscopy

A   B
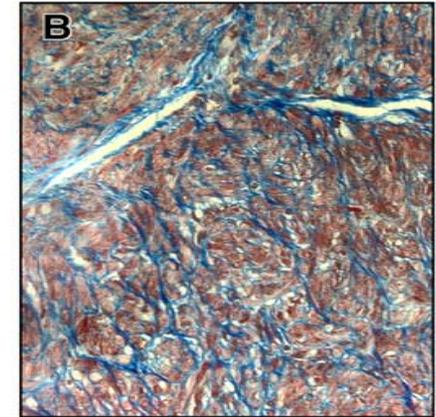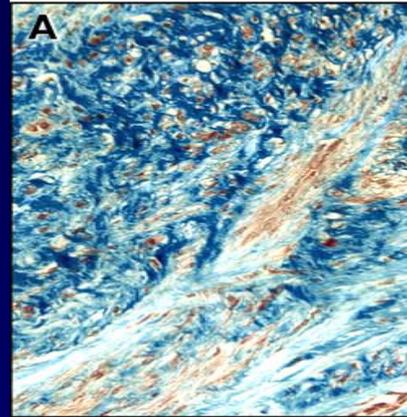
Polarized Light Microscopy

C   D

Electron Microscopy

E   F

Collagen Fibril Orientation

Myometrium

Leiomyoma

Myometrium
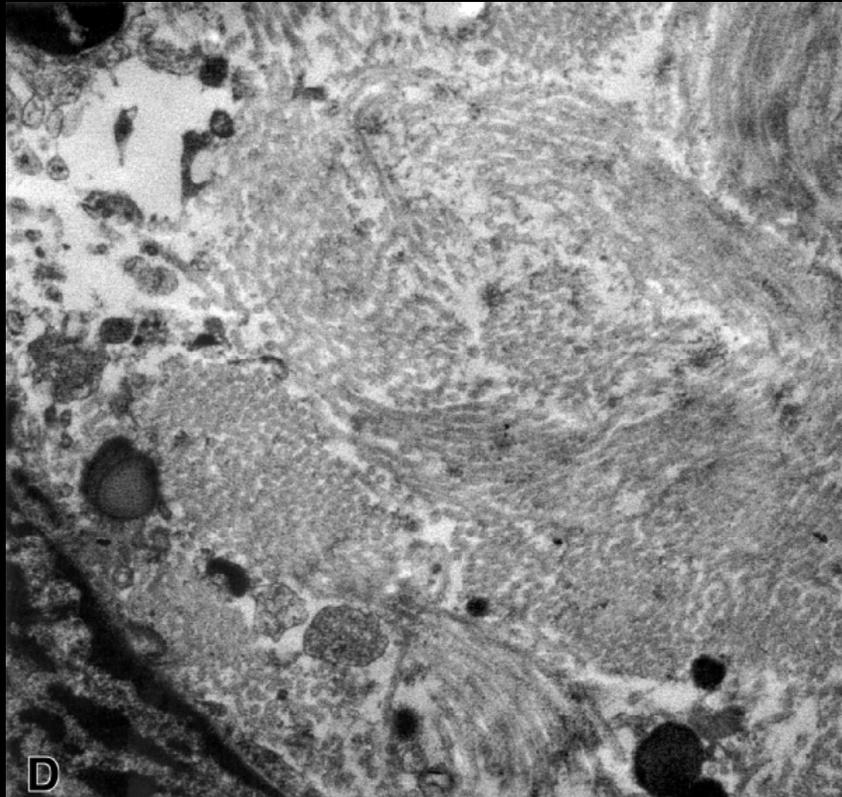
Leiomyoma
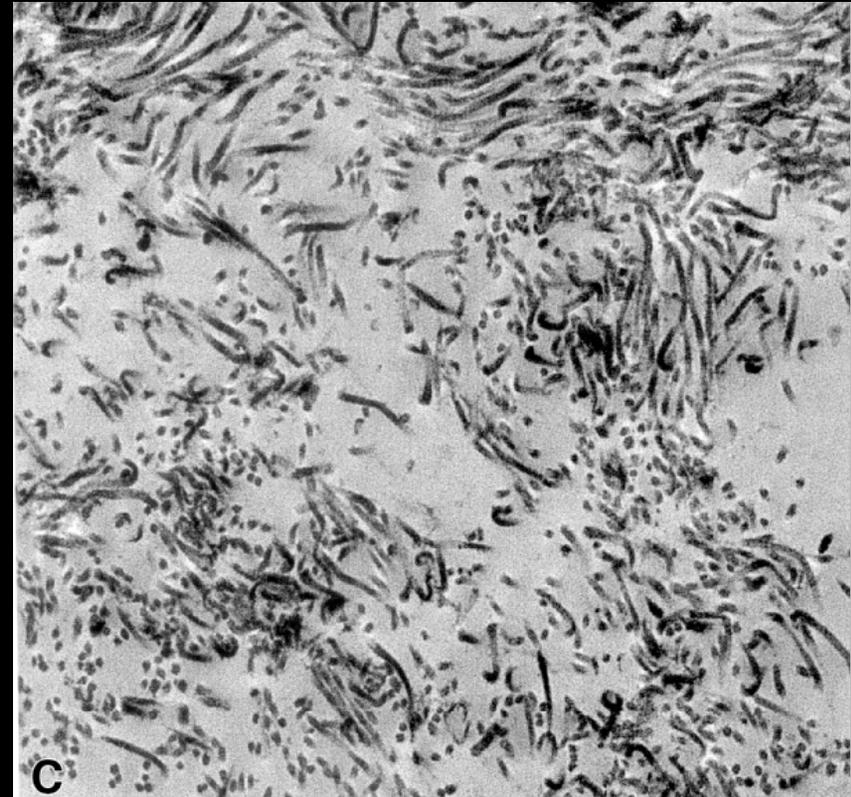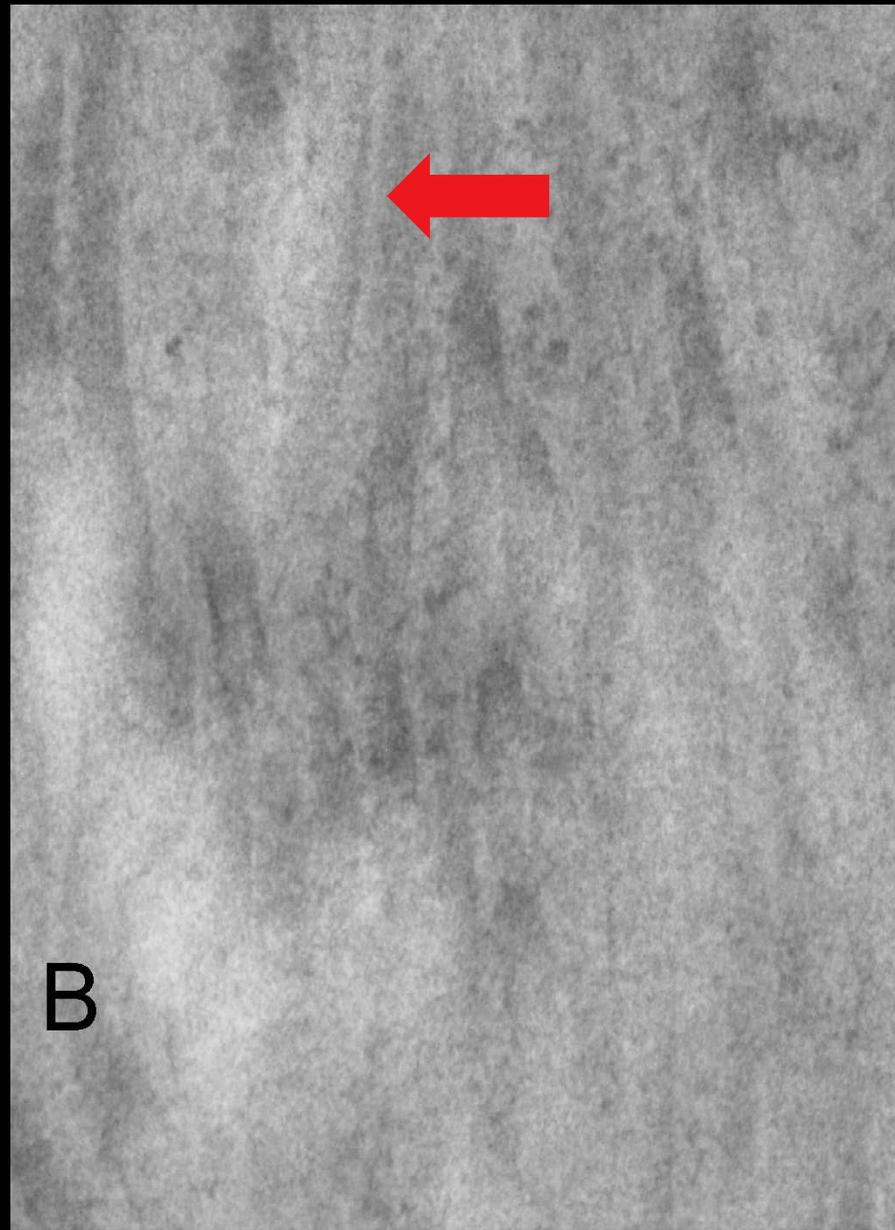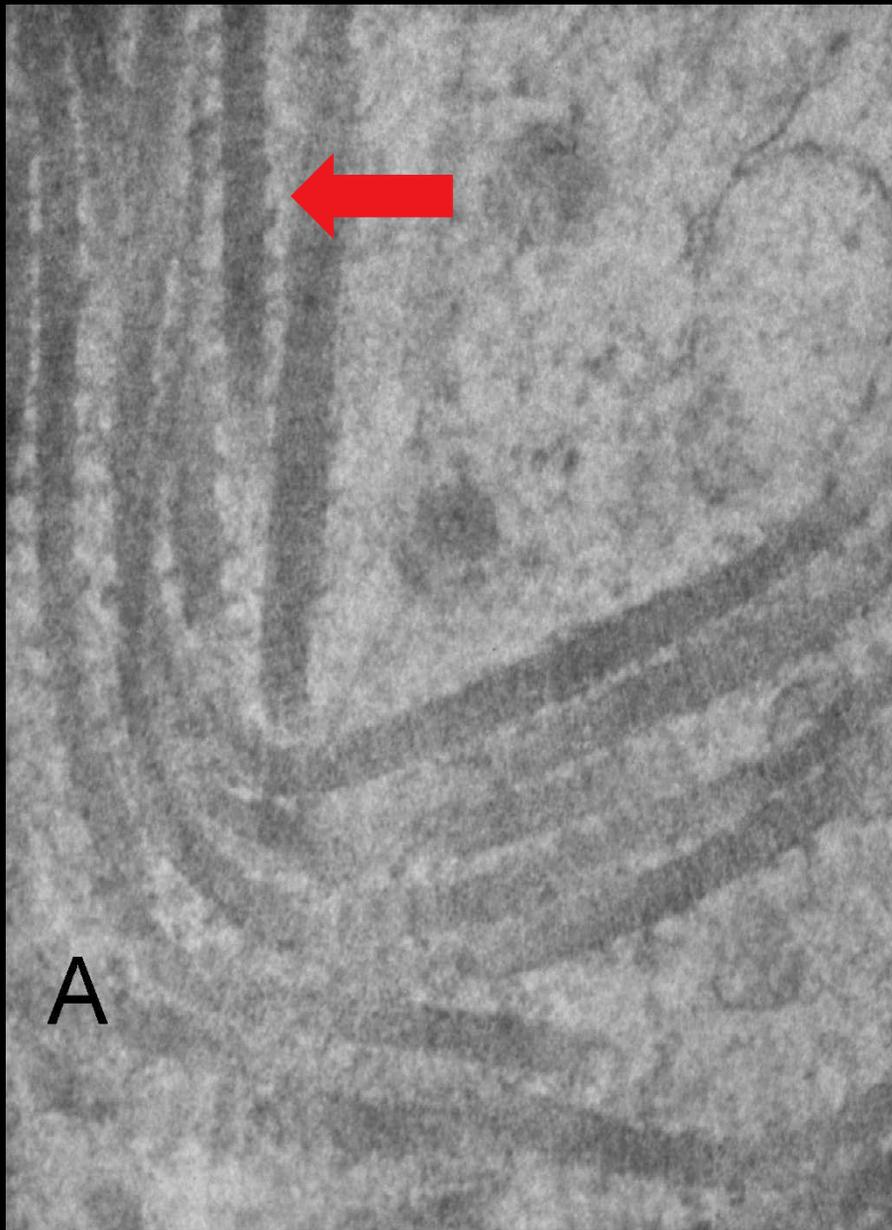
Heterotypic Fibril
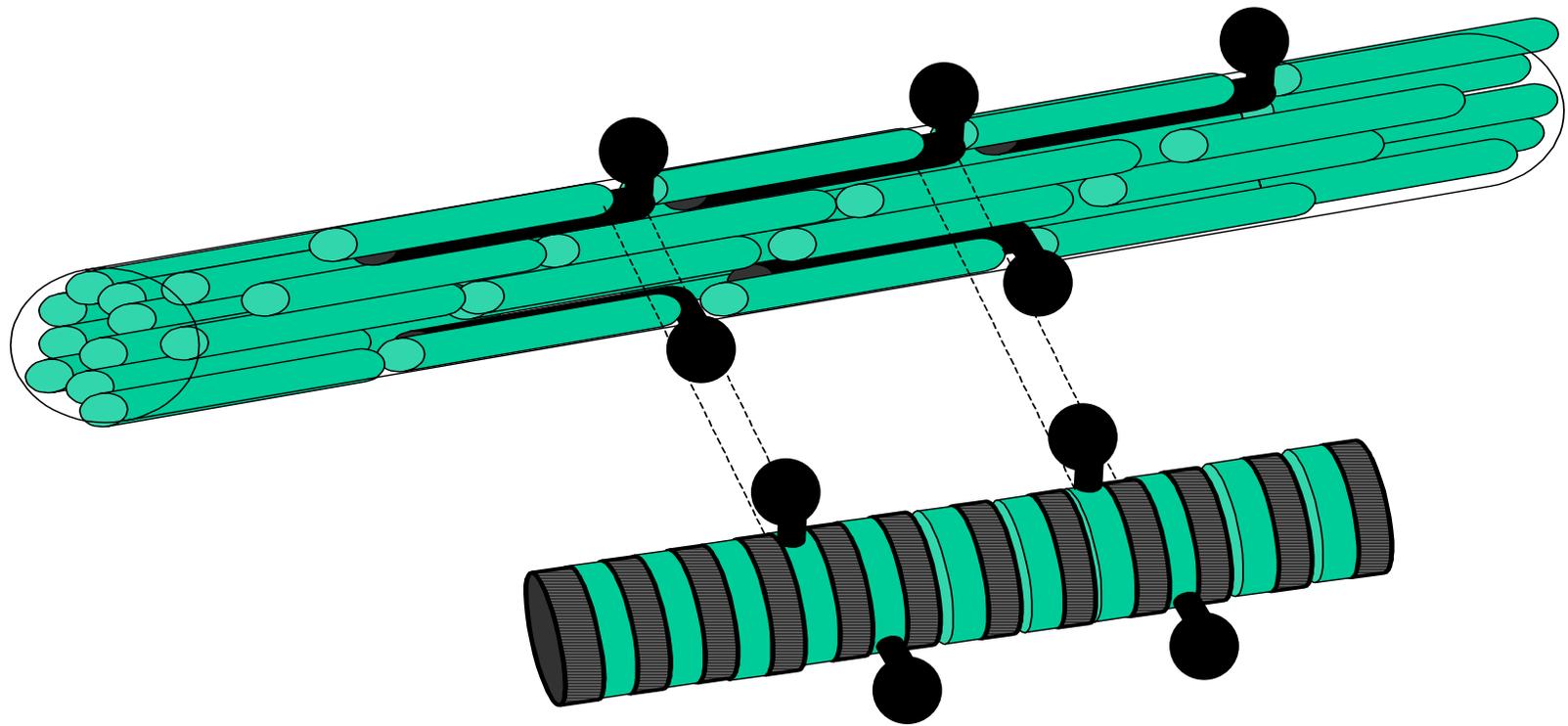
Leppert, et al. Fertil. Steril., 2004

Comparison of differentially expressed genes in fibroids

- **Under-Expressed Genes**
  - *Dermatopontin*
  - Alcohol Dehydrogenase
  - Prostaglandin E Receptor
- **Over-Expressed Genes**
  - CD24
  - CRABP II
  - IGF II

*Catherino, et al. Sem. Reprod. Med., 2004.*

Dermatopontin

- 22 kDa extracellular protein
- Binds the collagen-binding protein decorin
- Promotes cell adhesion and collagen formation
- Influences TGFβ and integrin cell signaling
- Reduced in Hypertrophic Scar

DERMATOPONTIN EXPRESSION

## A. Real-time RT-PCR

	Fold change	F/M	SEM
Mean	0.11 (-9.41)		[0.09,0.13]

## B. RT-PCR

Myometrium **Keloid** **Fibroid**

Skin **Liver**
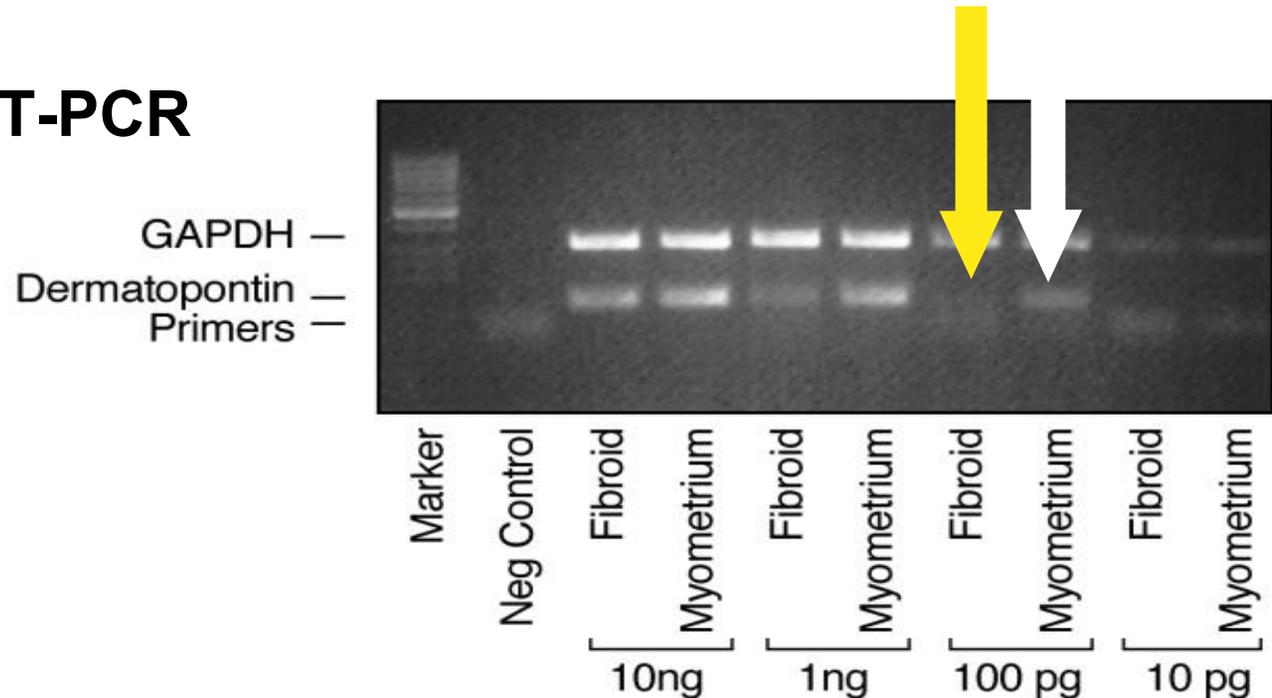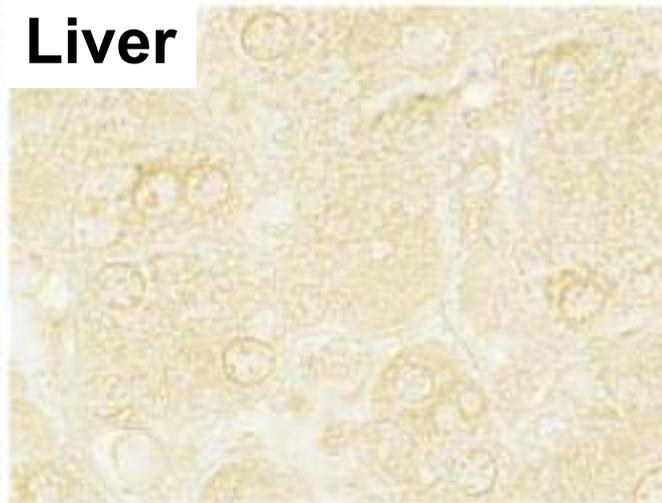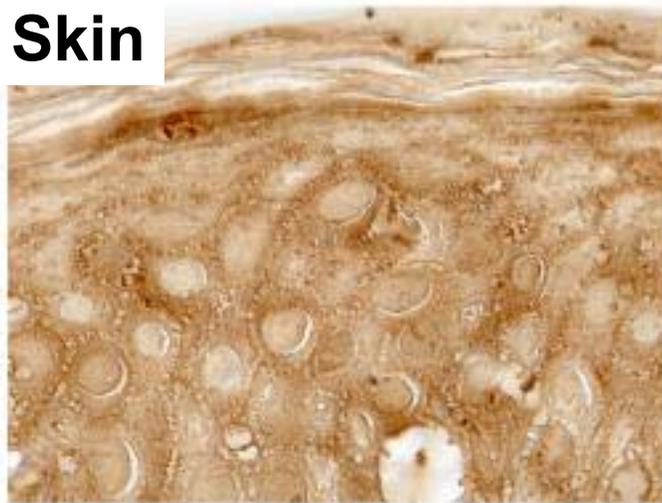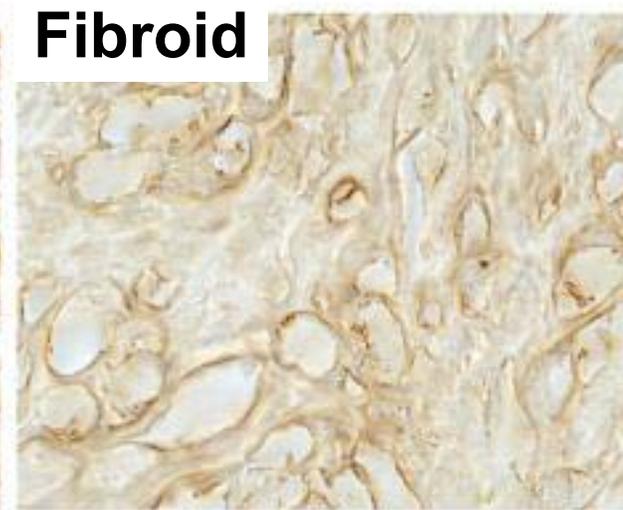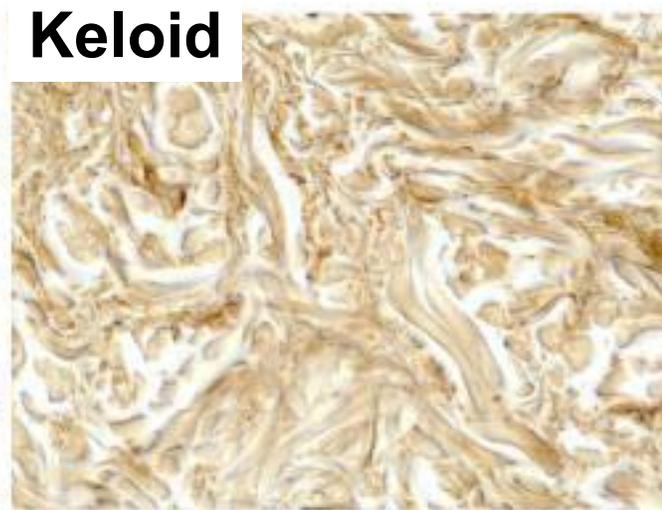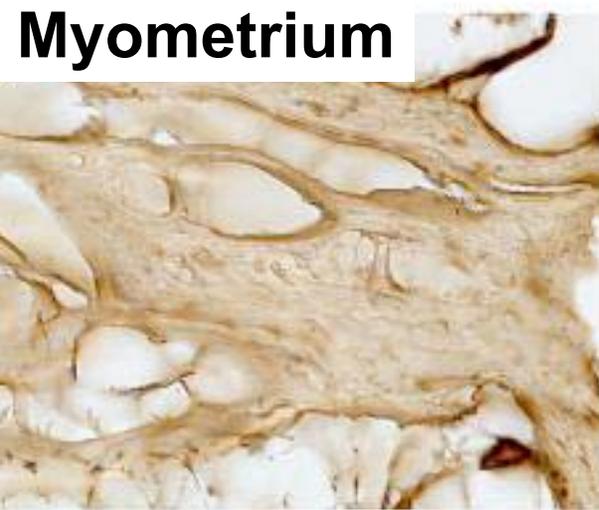
Anti-Dermatopontin (100X)

Catherino, et al. Genes, Chromosomes & Cancer, 2004.

Myometrium **Keloid** **Fibroid**

Skin **Liver**
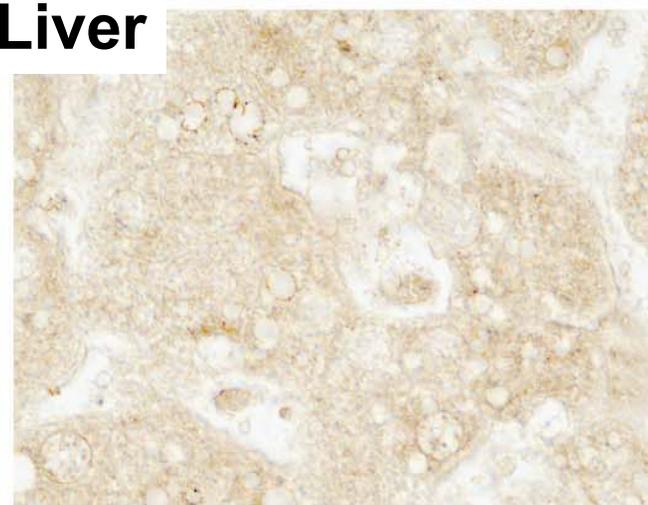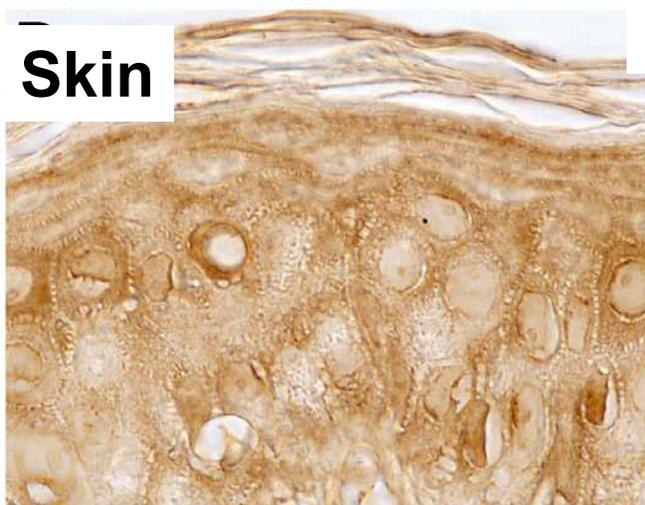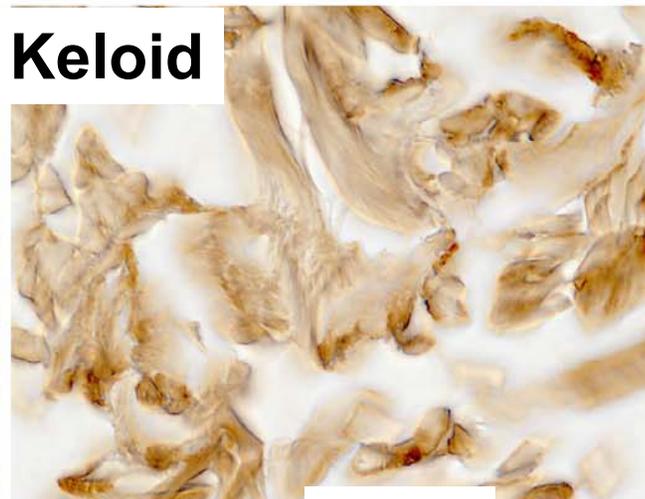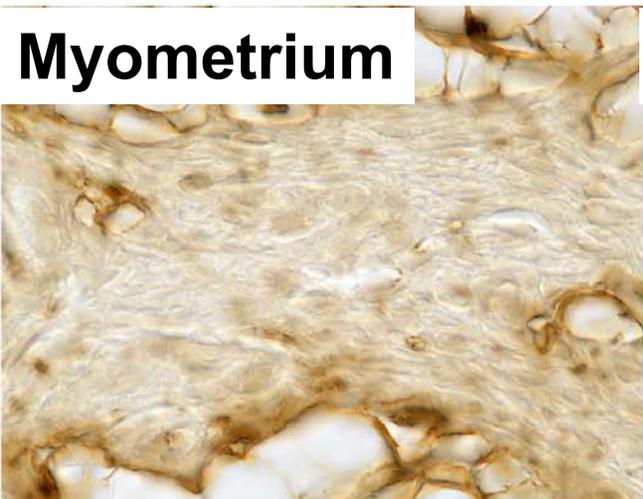
Anti-Decorin Staining (100X)

**Nuer Girl from Sudan with Keloid scarification**

Three Thousand year old sculpture

**Keloid**
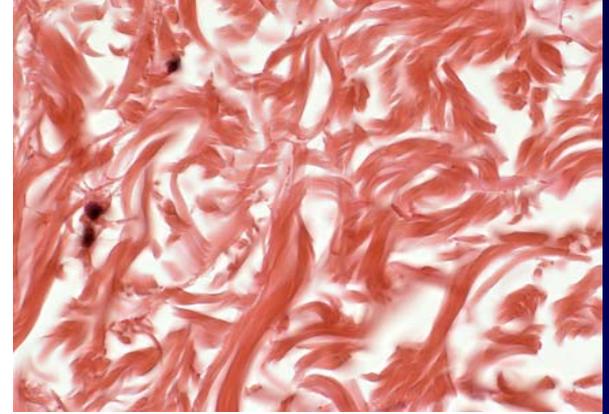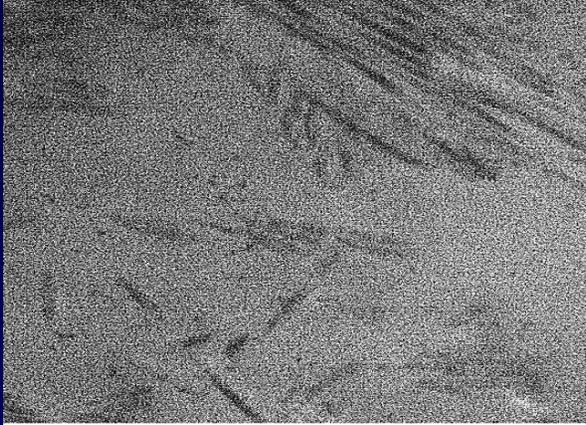
Fibroid
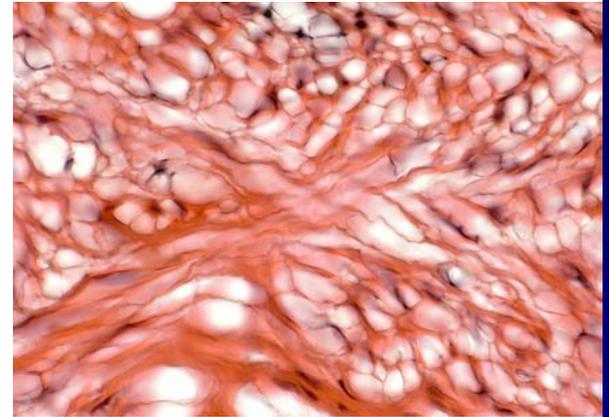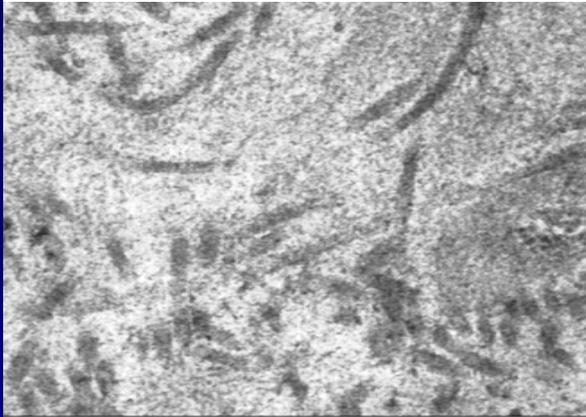
Myometrium
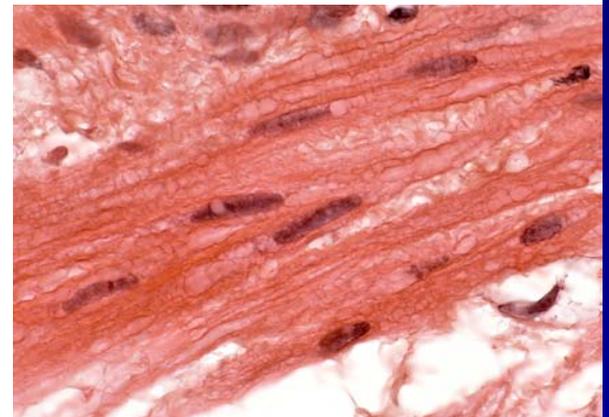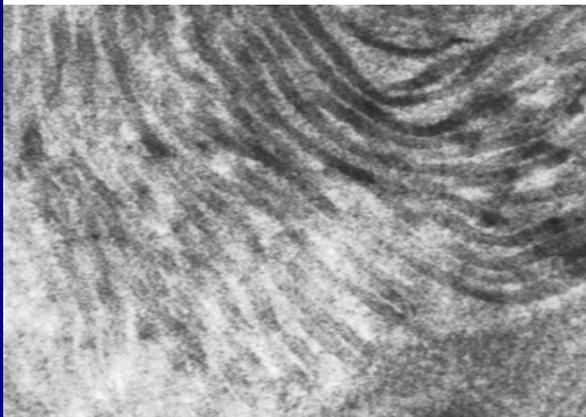
Electron Microscopy

Light Microscopy

Comparison of Gene Arrays

Gene	Leiomyoma[1]	Keloid[2]	similar?
Versican	5.79	7.74	√
COL I A2	-1.30	1.23	√
COL IV A6	-1.73	-1.64	√
TGFβ	β3 ↑	β1 ↑	

1. *Catherino, et al. Genes Chromo Cancer, 2004*
2. *Chen, et al. J Surgical Res 113:208, 2003*

Leiomyofibroblast:
Increased TGF expression, activation, receptor
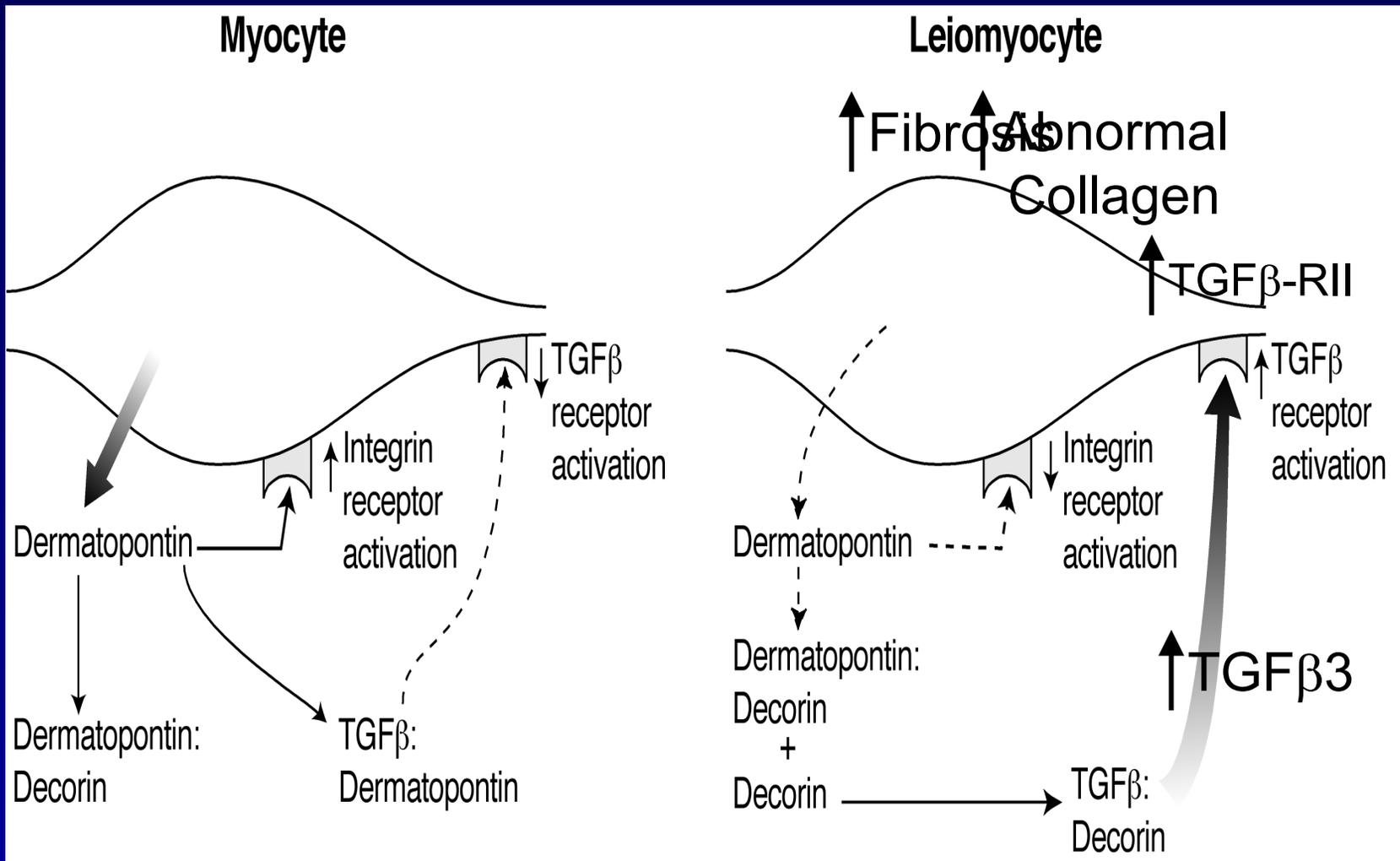
Leiomyofibroblast

1. Leiomyomata dysregulated ECM genes: ⬆TGFβ3 ⬆Versican ⬇DP

2. Disordered extracellular matrix: collagen fibril orientation

3. Not identical to lung, renal fibrosis

4. Similar disorder to Keloids?

Steps in wound healing:

1
inflammation
protomyofibroblast ➡ **2**
proliferation
myofibroblast ⫯➤ **3**
remodeling
apoptosis

leiomyoma and keloid cells remain in

proliferation stage

Possible New Strategies for intervention:

1. Collagenolytic: misoprostol

2. Signaling: rapamycin

3. Antifibrotic: pirfenidone

Pirfenidone:

1. Hermansky-Pudlak (pulmonary fibrosis)

2. Preliminary studies in leiomyoma

3. Current plans at NIH

Conclusions:

1. TGFβ plays a key role in development.

2. The ECM and mature collagen is abnormal.

3. Fibroids share many features with Keloids.

4. Pirfenidone holds promise as a nonsurgical, non-hormonal treatment for fibroids.

Acknowledgements
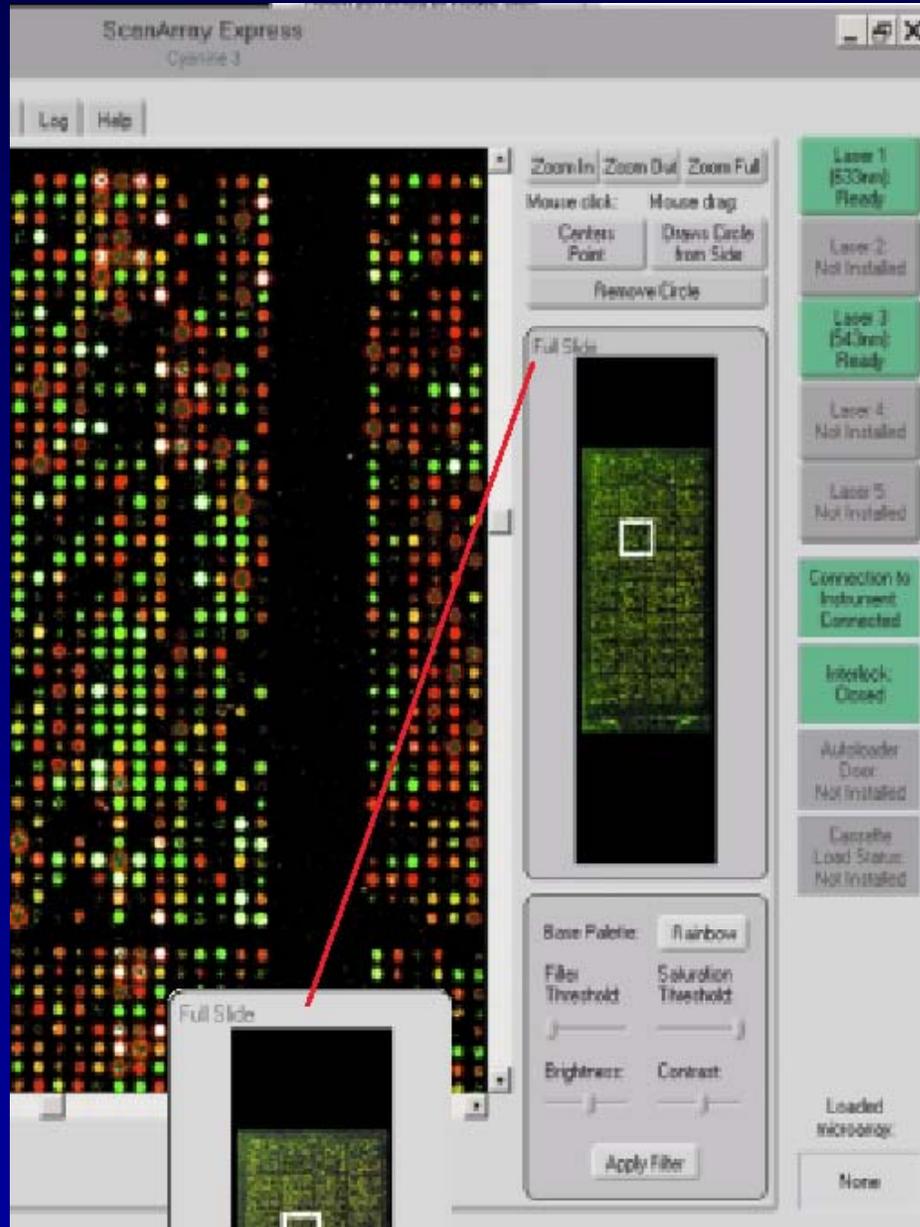
- **William Catherino, MD, PhD**
- **John C.M. Tsibris, PhD**
- **Phyllis Leppert, MD, PhD**
- **Lynnette Nieman, MD**
- **Cara Prupas, BA**
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- **Melinda Sharkey, BS**
- **Chantal Mayers, BA**
- **Minnie Malik, PhD**
- **Clariss Potlog-Nahari, MD**

Uterine Leiomyomata:
a public health problem

PEDUNCULATED

INTRACAVITARY

INTRAMURAL

SUBMUCOUS

SUBSEROUS
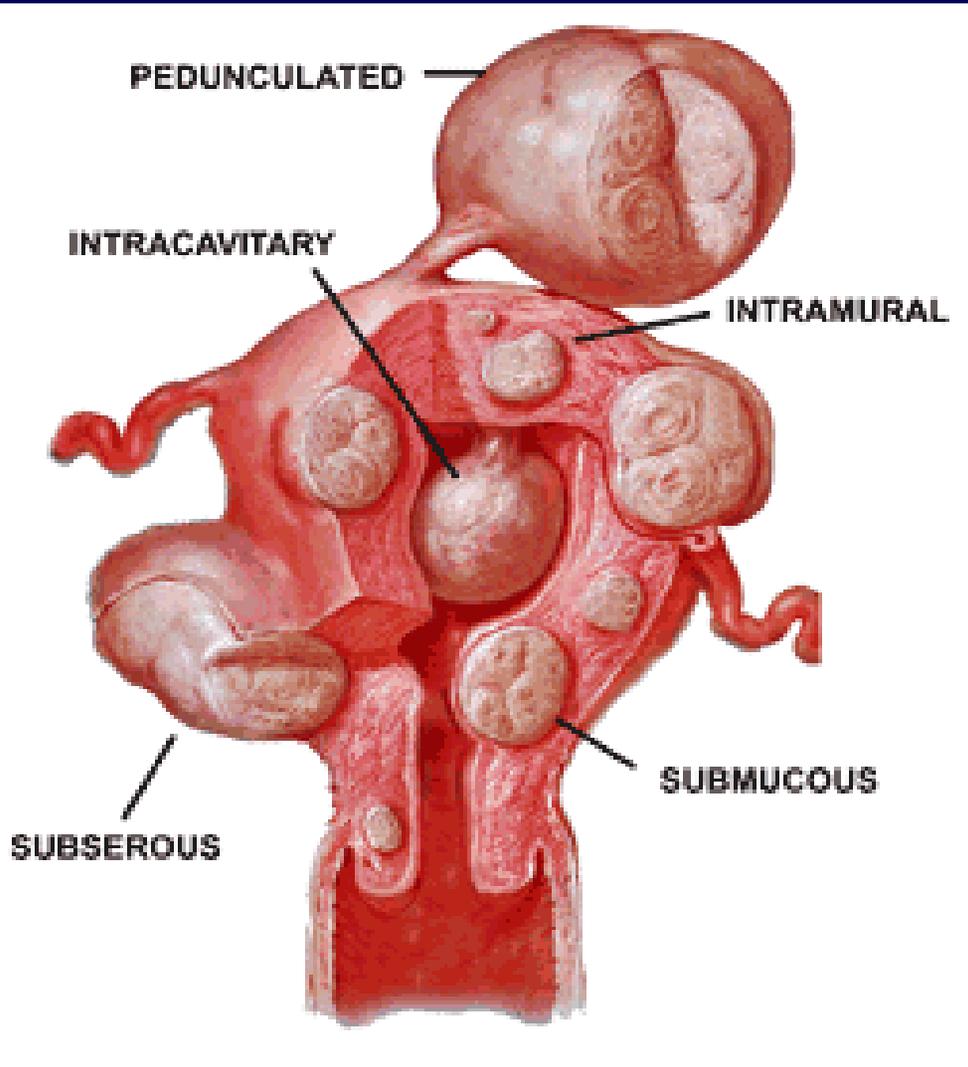
Incidence
25-50%

21% Pre-term labor
v.s. 3.9% controls

Pain

bleeding

anemia
= $ 2.3 billion in U.S.