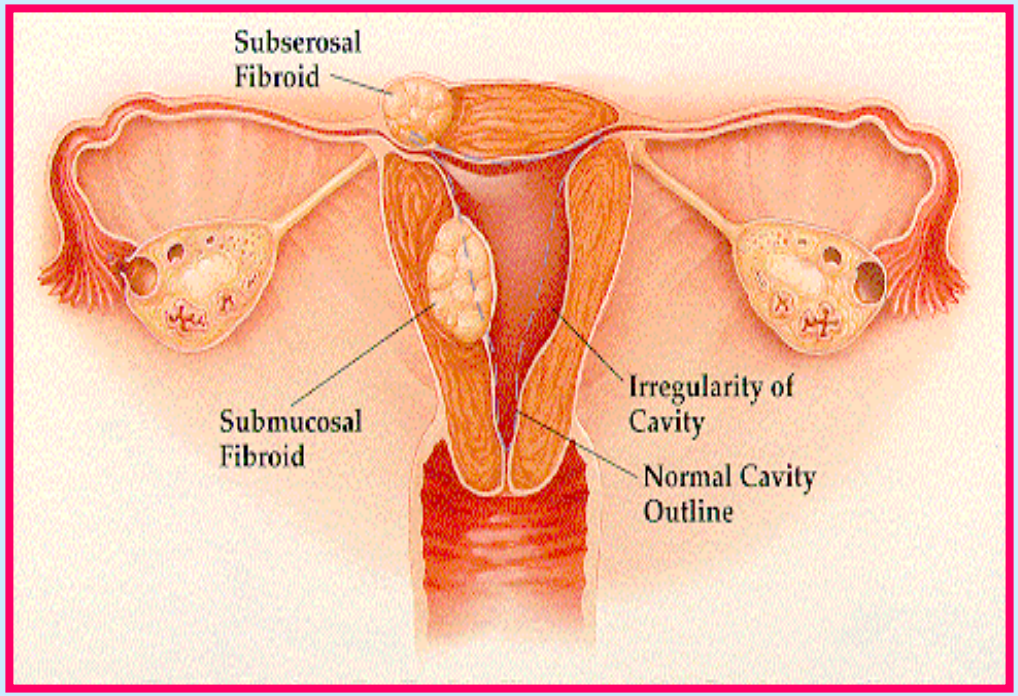
Signaling Pathways of Smooth Muscle Cells in Leiomyomas

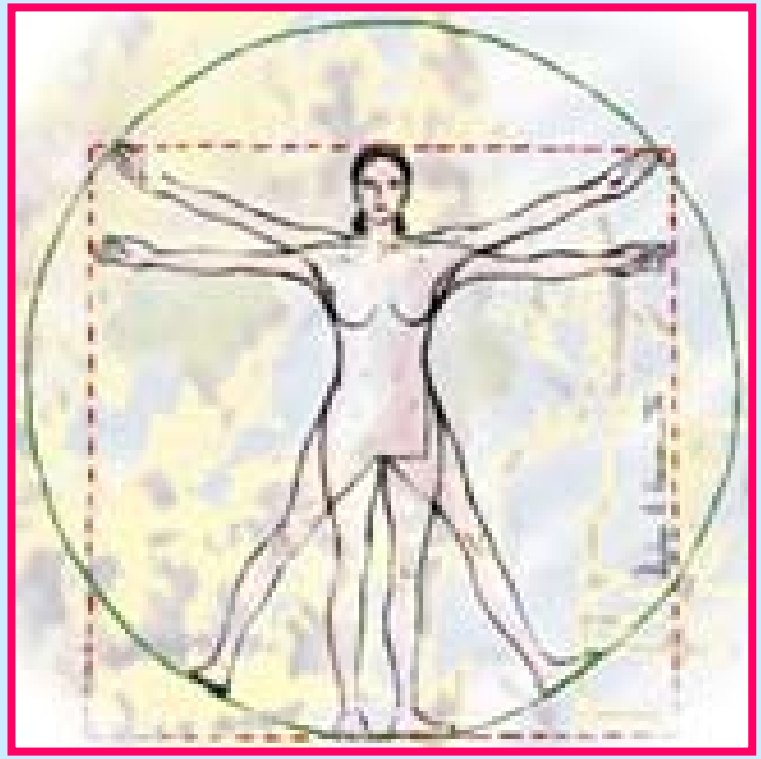


Romana Nowak, PhD
University of Illinois

UIC Center for Women's Health and Reproduction

Who is affected?

- Most common pelvic tumor in women, developing during reproductive years
- Reported symptomatic prevalence of 20-25%
- Menopause decreases symptoms and growth



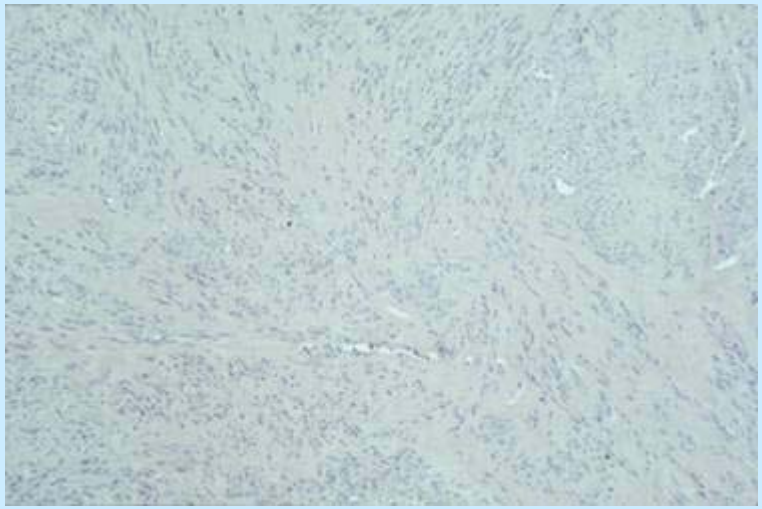
Detection of Uterine Leiomyomas, Reliability of Routine Pathology Reports

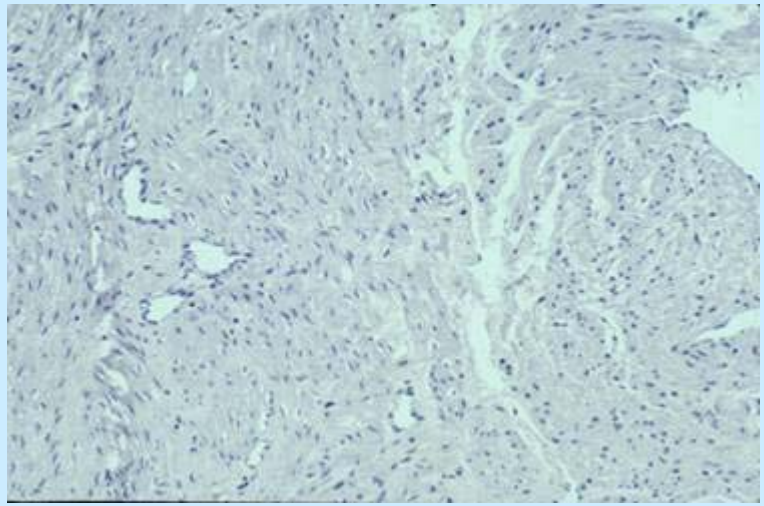
	Premenopausal	Postmenopausal	Total
Incidence of reported myomas	34/68 (50%)	18/32 (56%)	52/100
Incidence of myomas after gross and microscopic case review and gross serial sectioning	50/68 (74%)	27/32 (84%)	77/100
Average number of myomas	7.6	4.2	6.5
Average size of largest myoma (mm)	18.8	11.5	16.5

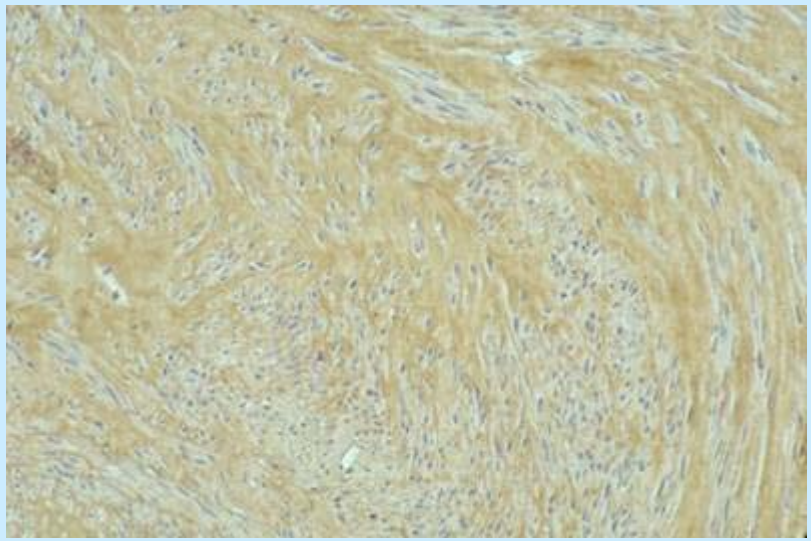
Cramer and Patel, 1990

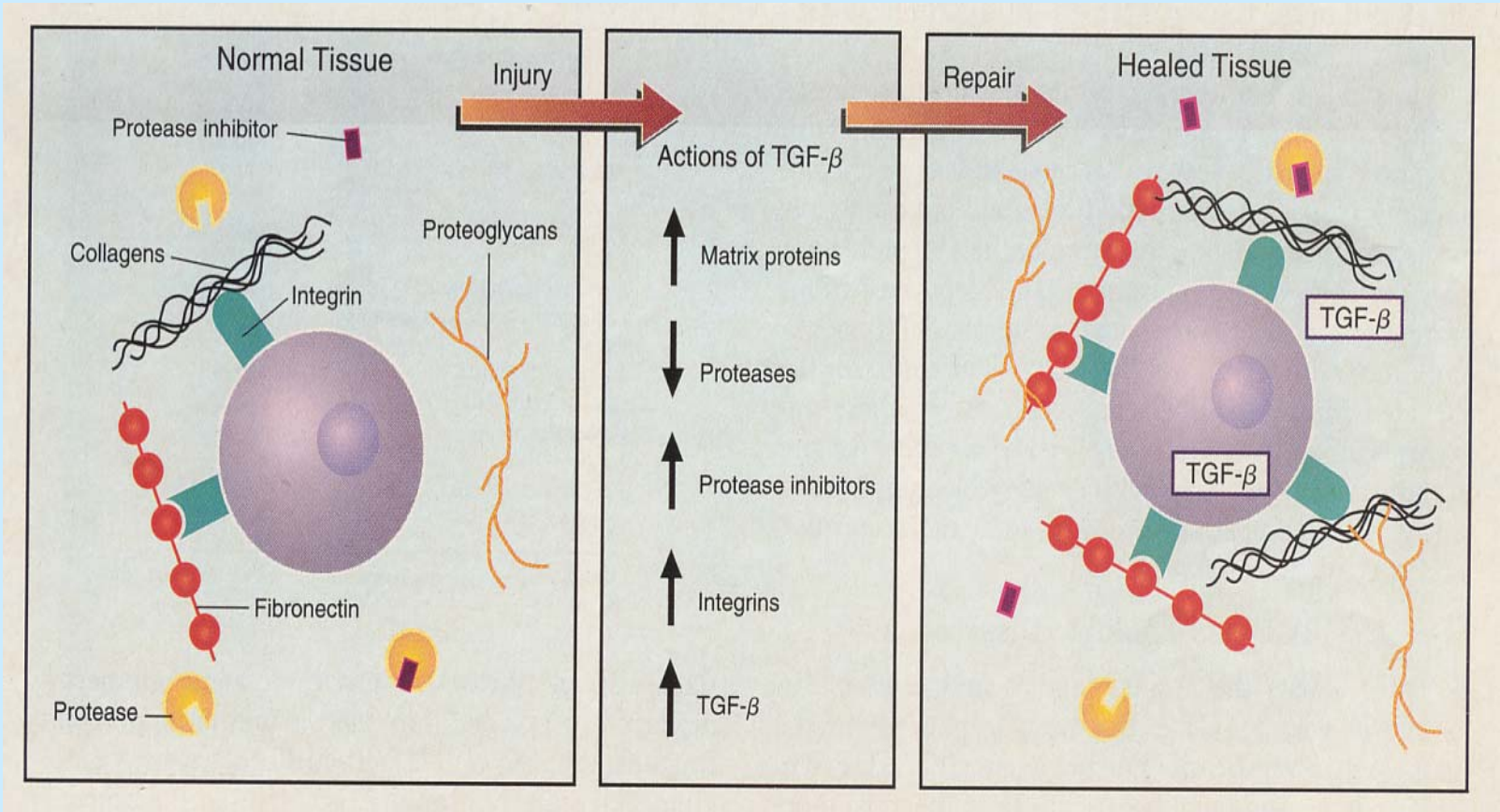
Epidemiology

- Increased occurrence (3-9 times higher) in African-American women vs. Caucasian women
- Higher risk among women with a family history of fibroids
- Risk factors for symptomatic fibroids:
    * high BMI (obesity)
    * diabetes
    * hypertension
    * other benign fibrotic conditions, such as renal fibrosis

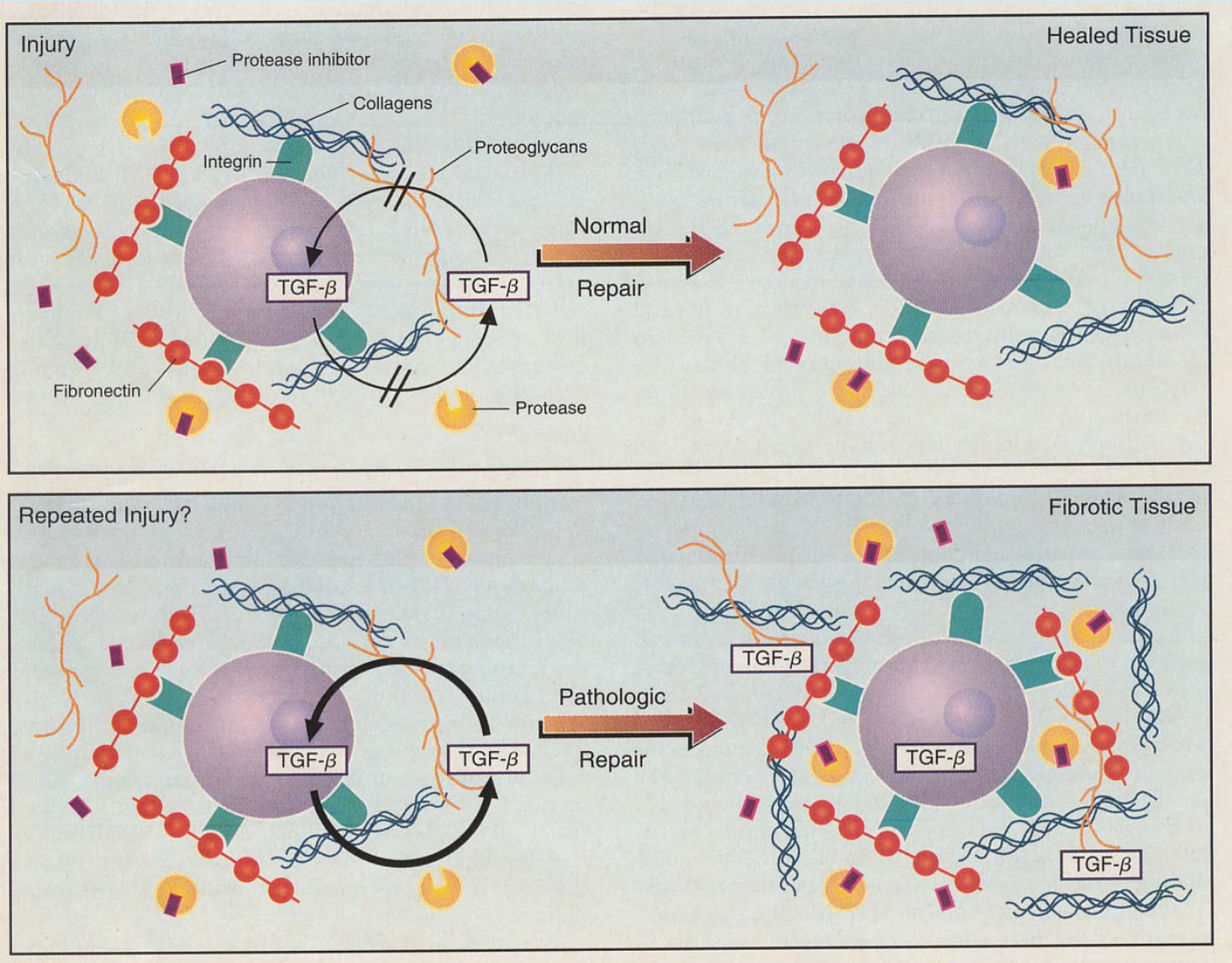








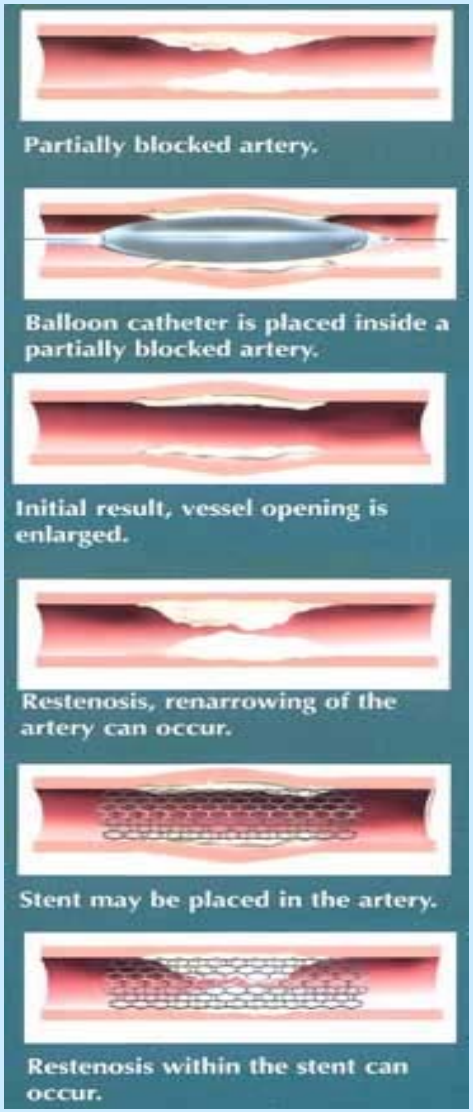
Border and Noble, 1994



Border and Noble,1994

Vascular SMCs vs Uterine Myometrial SMCs

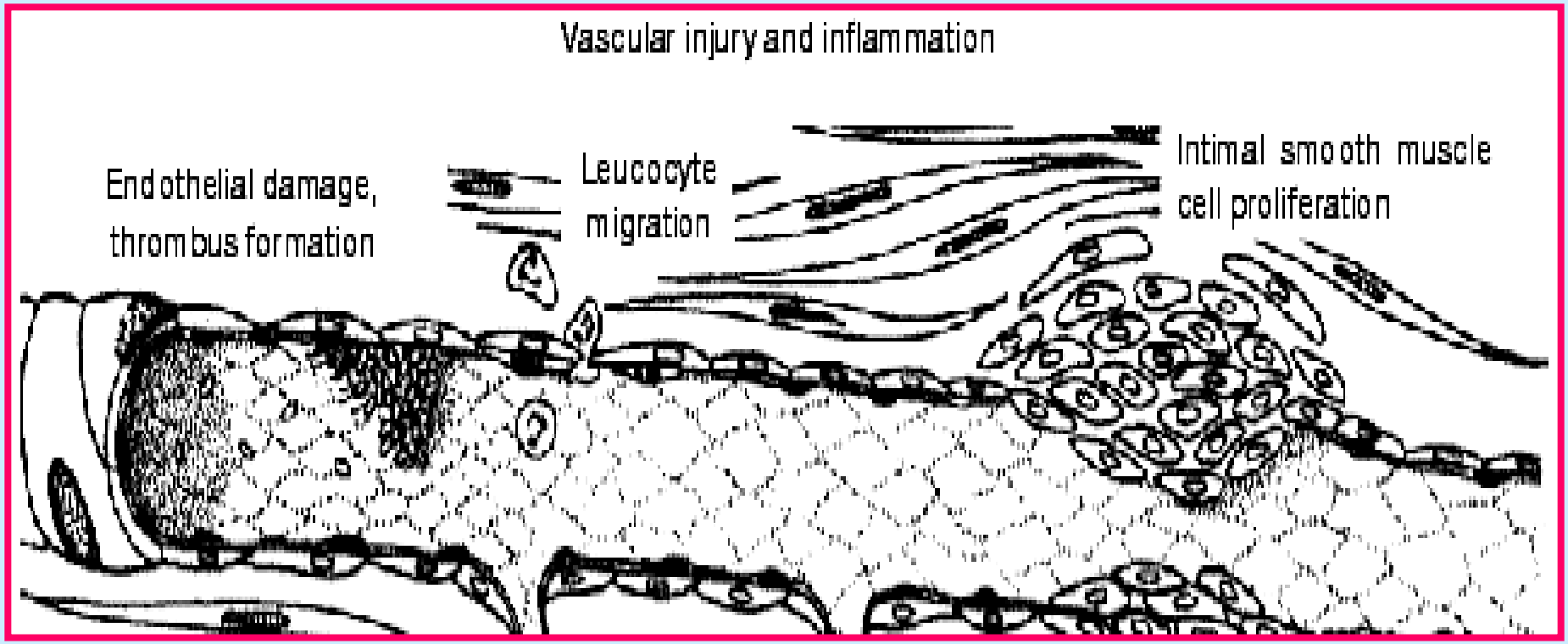
- Respond to similar growth factors
- Both are typically quiescent, exhibit little proliferation
- Similar response to injury? → increased proliferation of smooth muscle cells and excessive collagen production



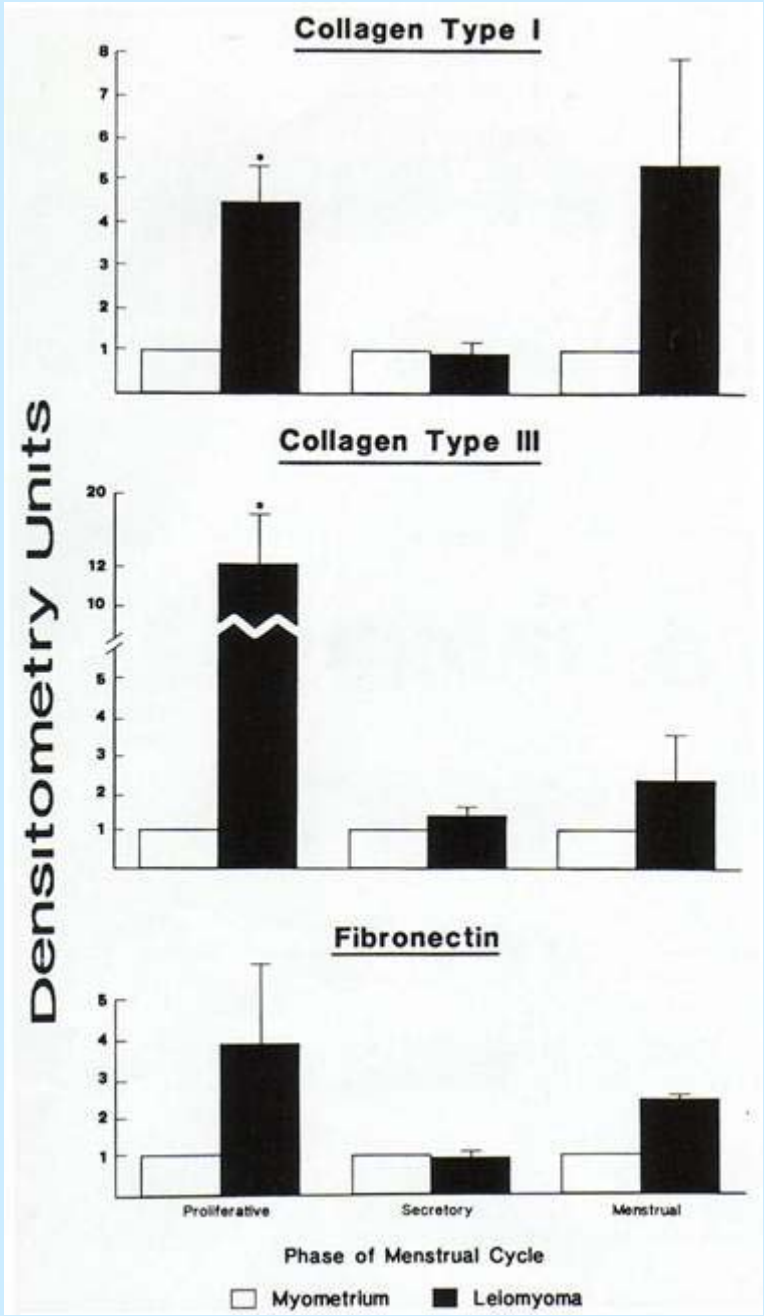
Injury Response Model

Restenosis

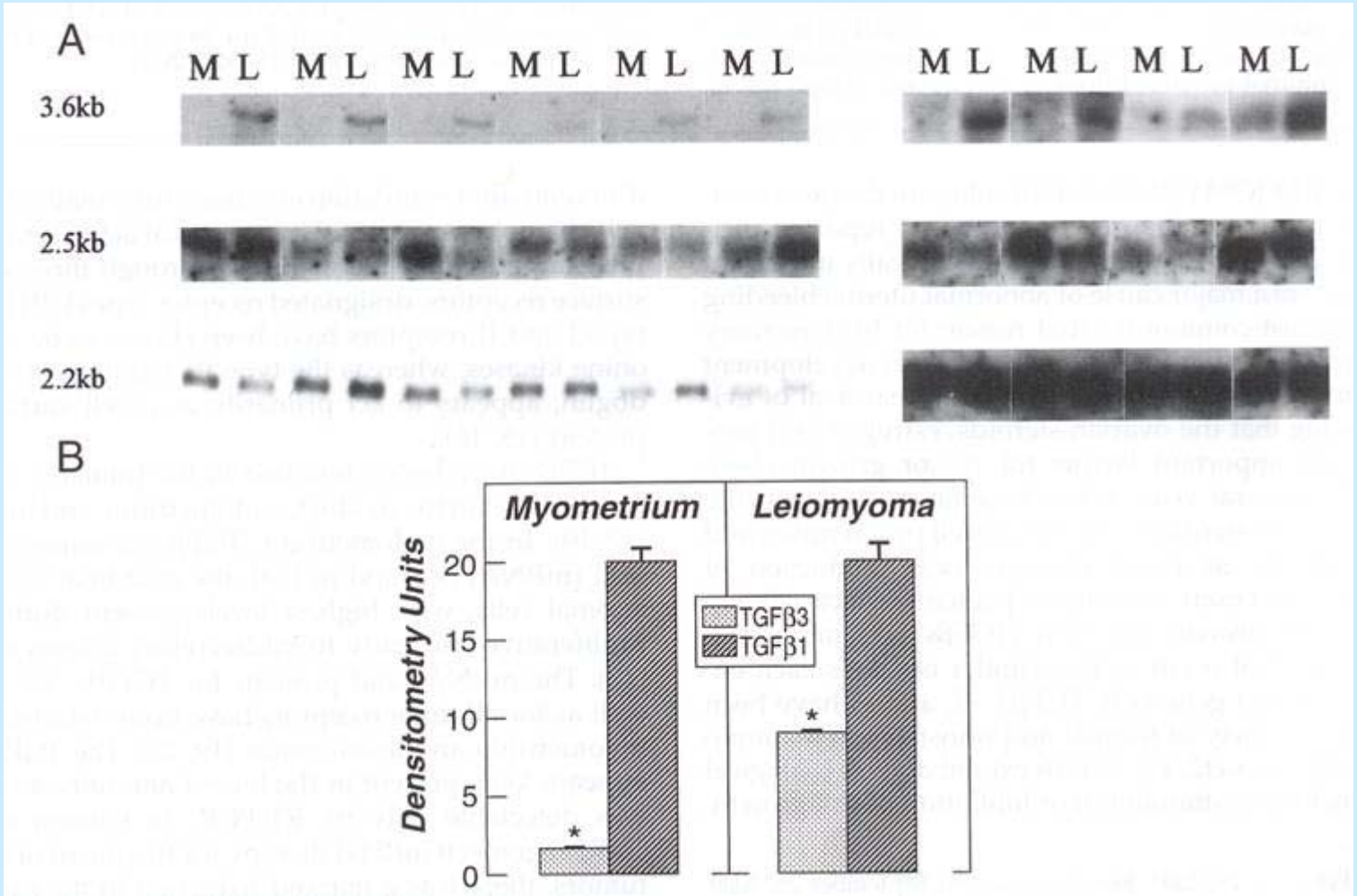
Fibroid development may be caused by a uterine smooth muscle cell response to injury, just as restenosis results from injury to vascular smooth muscle cells.



In response to injury, VSMCs proliferate and migrate into vessel, induced by growth factors such as EGF and PDGF



Stewart et al., 1994



Lee & Nowak, 2001

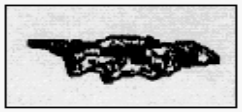
Normal Myocyte

Injury

(Hypoxia, bacteria)

Proliferation

Genetic Mutation



Mutated Myocyte

Ovarian Steriods

Growth factor production
Expression of growth factor receptors
Extracellular matrix production
Mitogenesis

Angiotensin II
Oleic Acid
Insulin
et al.

Clonal Expansion

Myoma

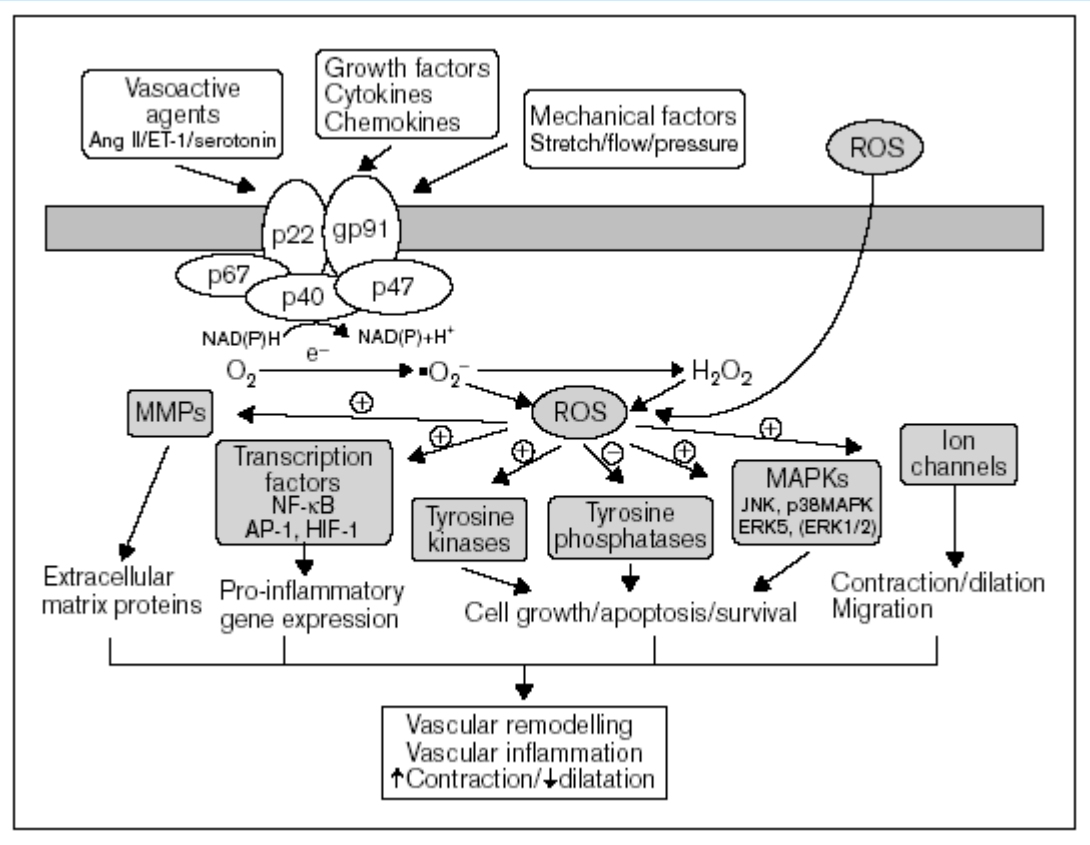
Reactive Oxygen Species (ROS)

- Second-messenger molecules
- Activate variety of tyrosine kinases, altering signaling pathways that mediate cellular growth, apoptosis, and migration
- ROS activates EGF and PDGF receptor tyrosine kinases
- Addition of PDGF produces rapid rise in ROS levels in vascular smooth muscle cells (Sundaresan et al., 1995)

Includes…

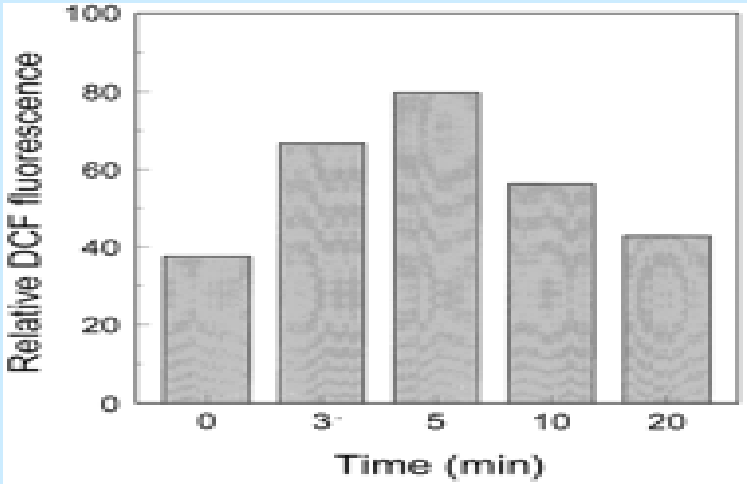
- Hydrogen peroxide (H_2O_2)
- Superoxide ($\bullet O_2^-$)
- Hydroxyl ($\bullet OH$)
- Hydrochlorous acid (HOCl)
- Ozone (O_3)

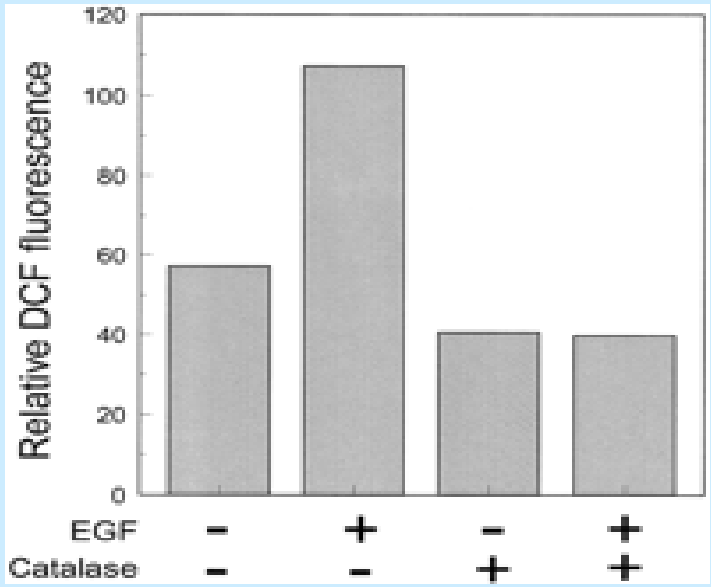
Reactive Oxygen Species Signaling Pathway



Touyz, 2003

EGF Induces ROS Production in Fibroblast Cells



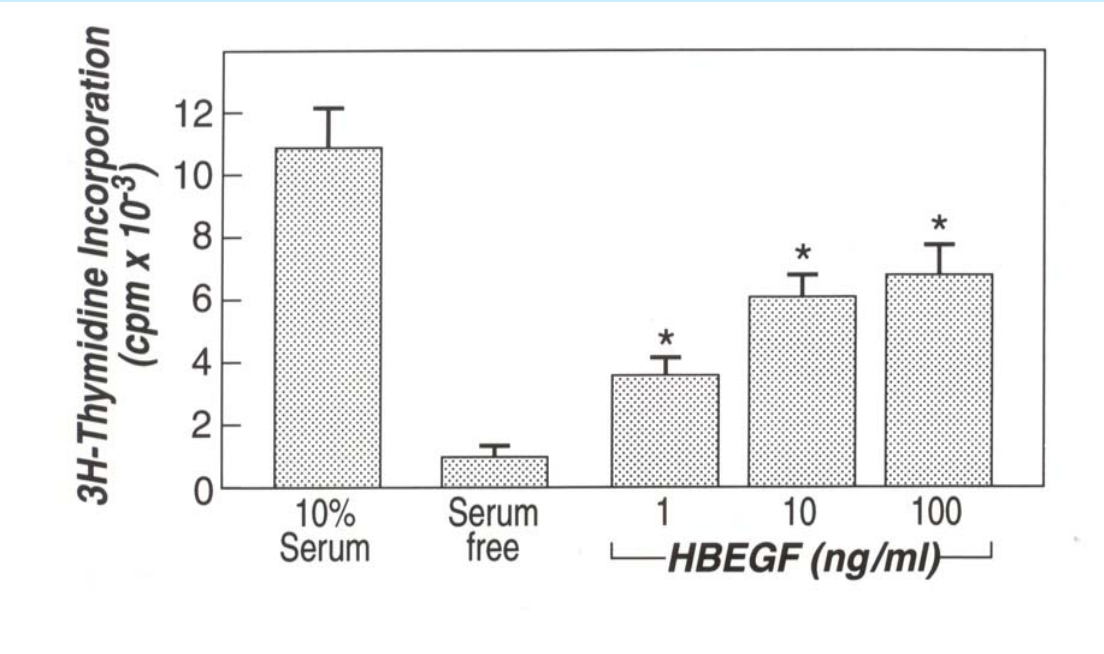


Bae et al., 1997

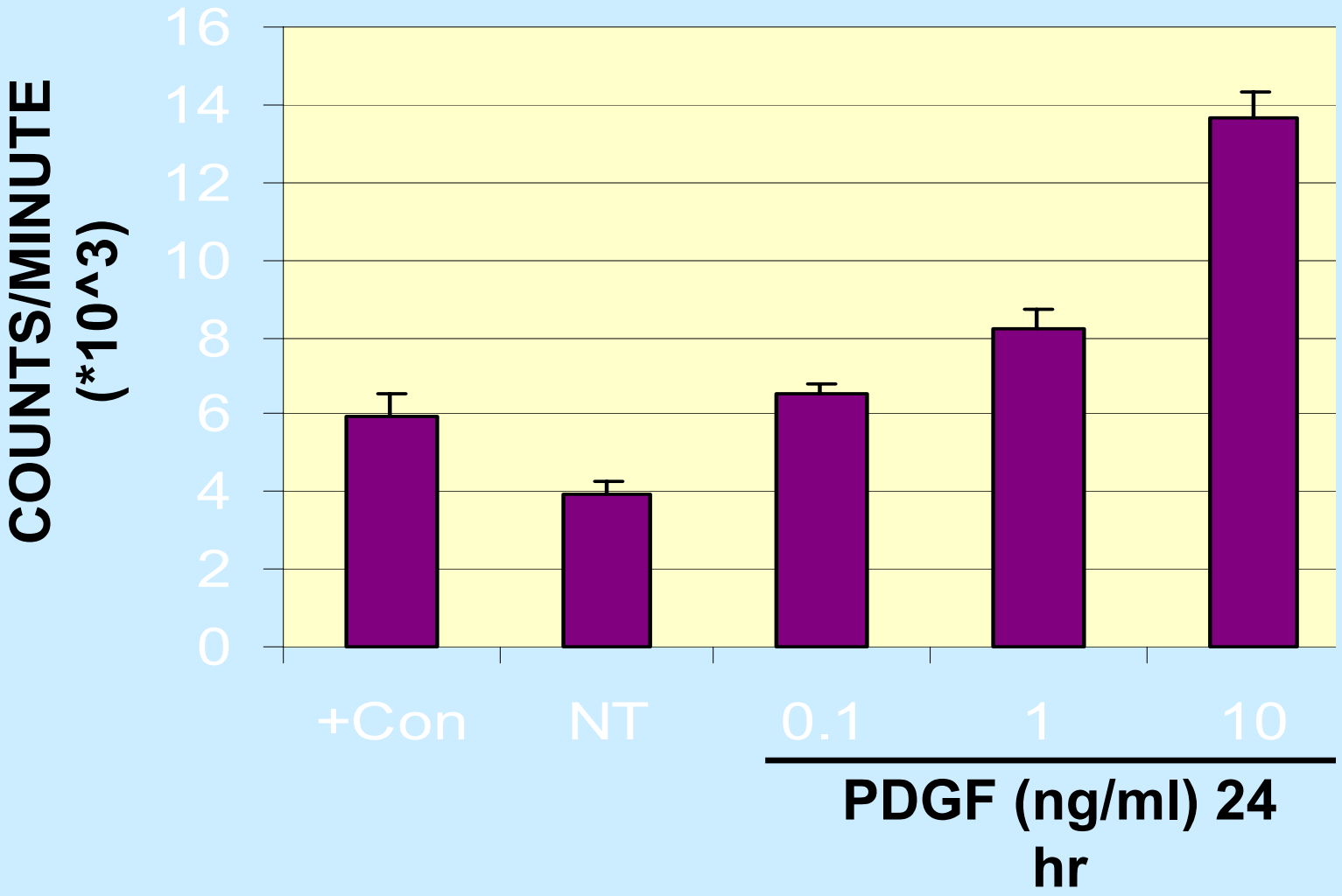
Hypothesis

ROS are necessary components of the EGF and PDGF signaling pathways that regulate proliferation and matrix production by leiomyoma SMCs

Effects of EGF/HBEGF on Proliferation of Leiomyoma SMCs



Thymidine Assay
Effects of PDGF on Fibroid cell Proliferation

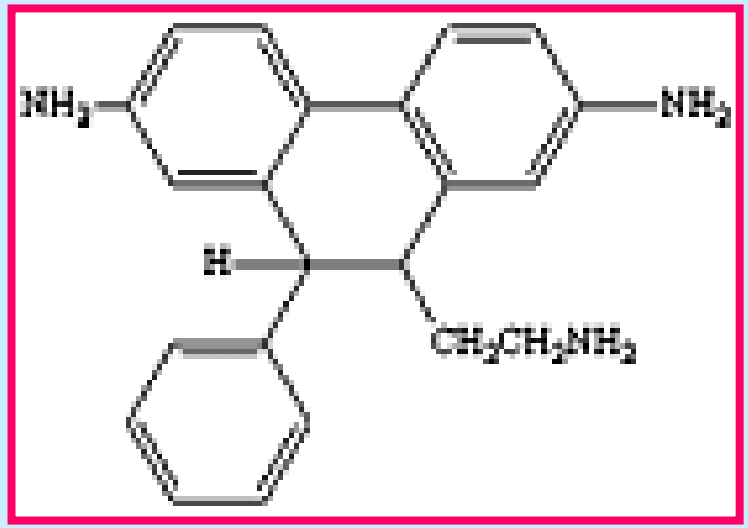


Effects of EGF/PDGF on intracellular ROS levels, Protocol

- Cells washed with DMEM, then treated with varying concentrations of EGF and PDGF for increasing time increments (0, 5, 10, 15, 20, or 30 minutes)
- Remove growth factor treatment, then add 10uM DHE fluorescent dye (loading time = 20 minutes)
- Cells washed, then medium removed and picture taken
- Increase in fluorescence indicates an increase in ROS production

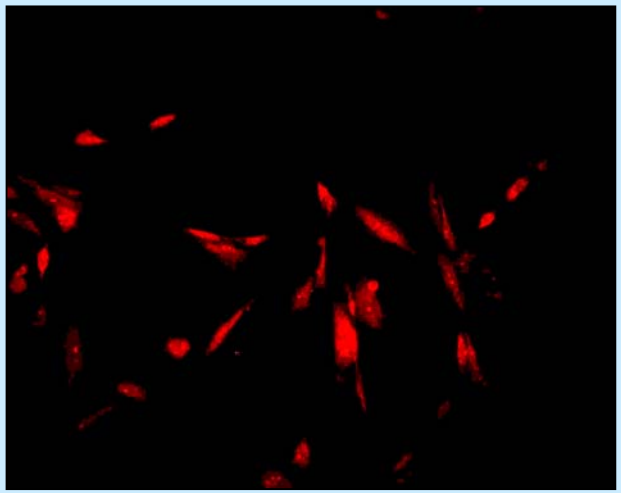
Dihydroethidium (DHE)

- Reduced form of ethidium bromide
- Non-fluorescent, can passively enter cell
- Upon re-oxidation by intracellular ROS, gives off a red fluorescence and is intercalated into the DNA
- Used to detect oxidative activities in cells

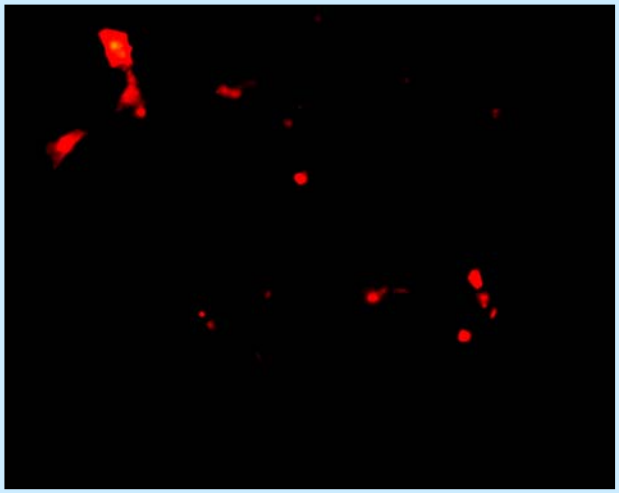


FLUORESCENCE INCREASES WITH ADDITION OF EGF or PDGF

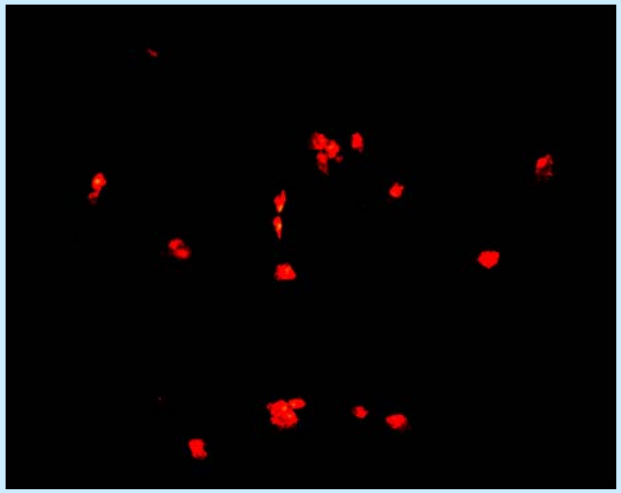
DMEM, T15



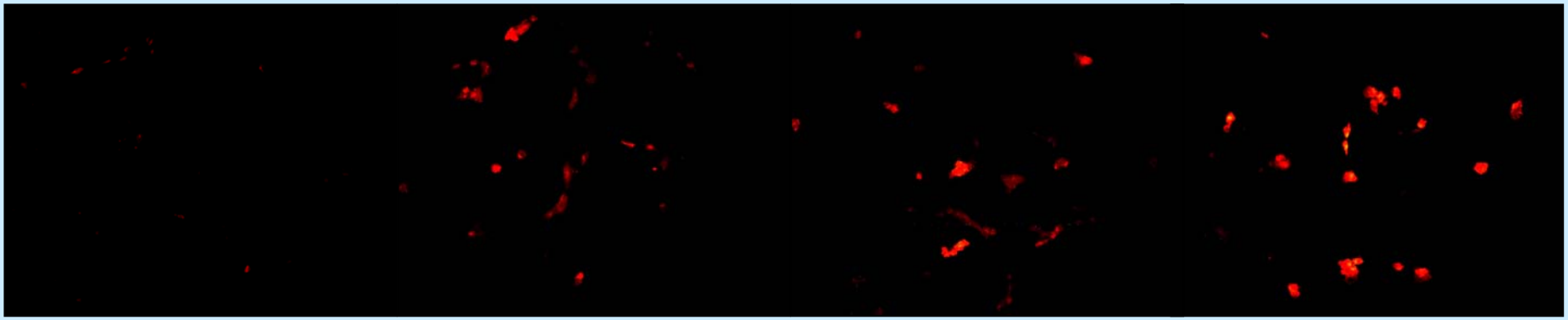
10ng/mL PDGF, T15



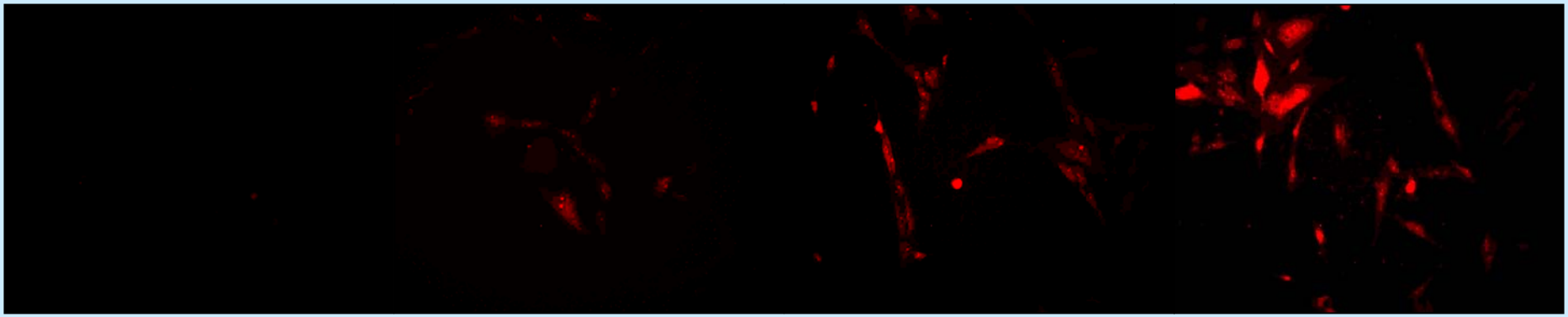
1mM H202, T15



100ng/mL EGF, T15



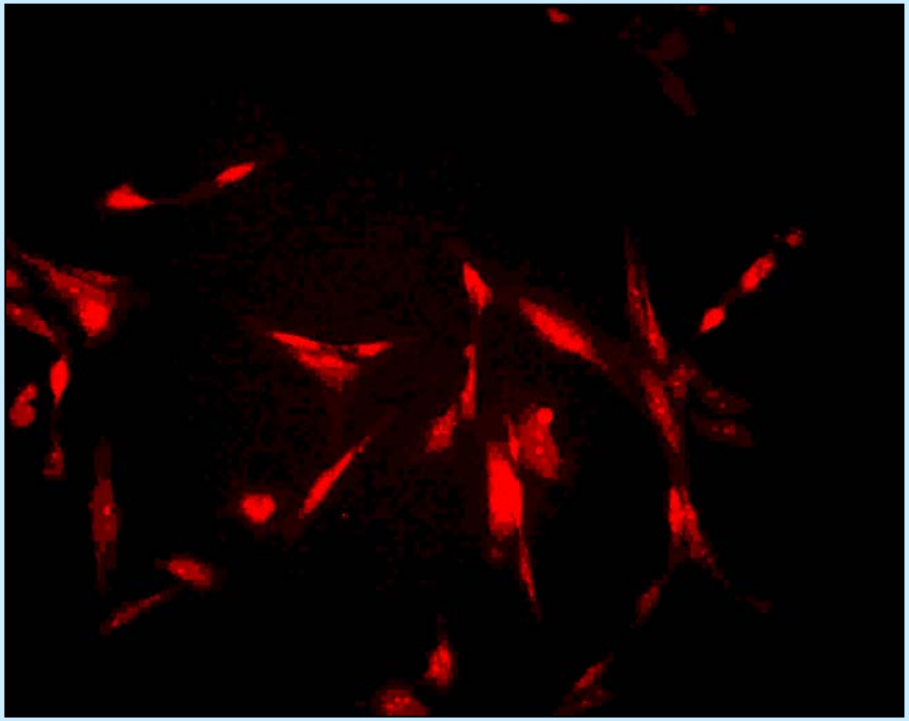
100 ng/mL EGF: T0, T5, T10, T15



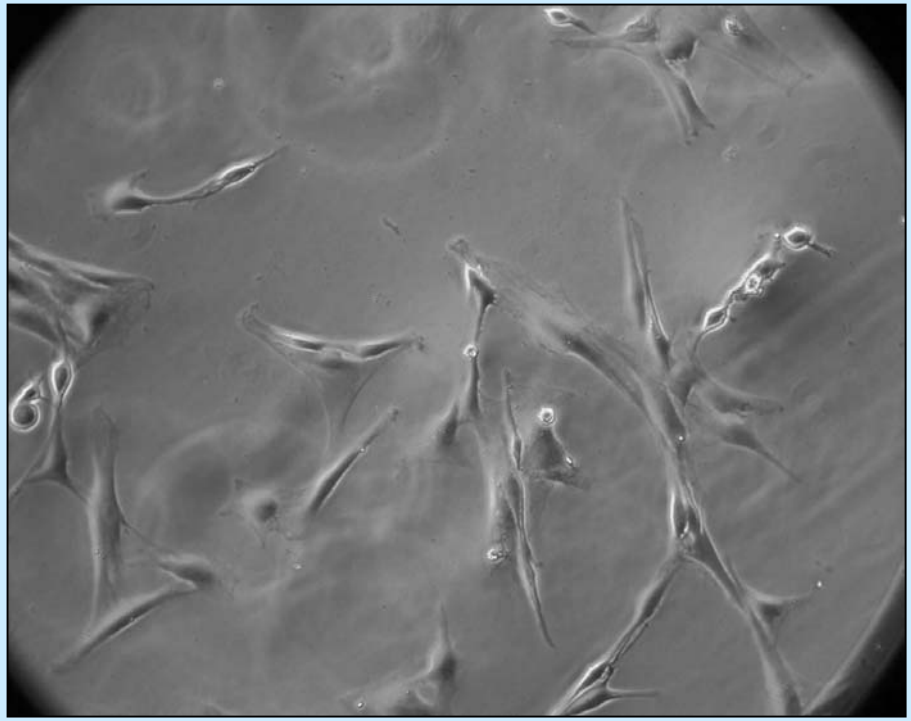
10ng/mL PDGF: T0, T5, T10, T15

Fluorescence increases as treatment period with PDGF or EGF increases

Myometrium (M106) 10ng/mL PDGF, T15

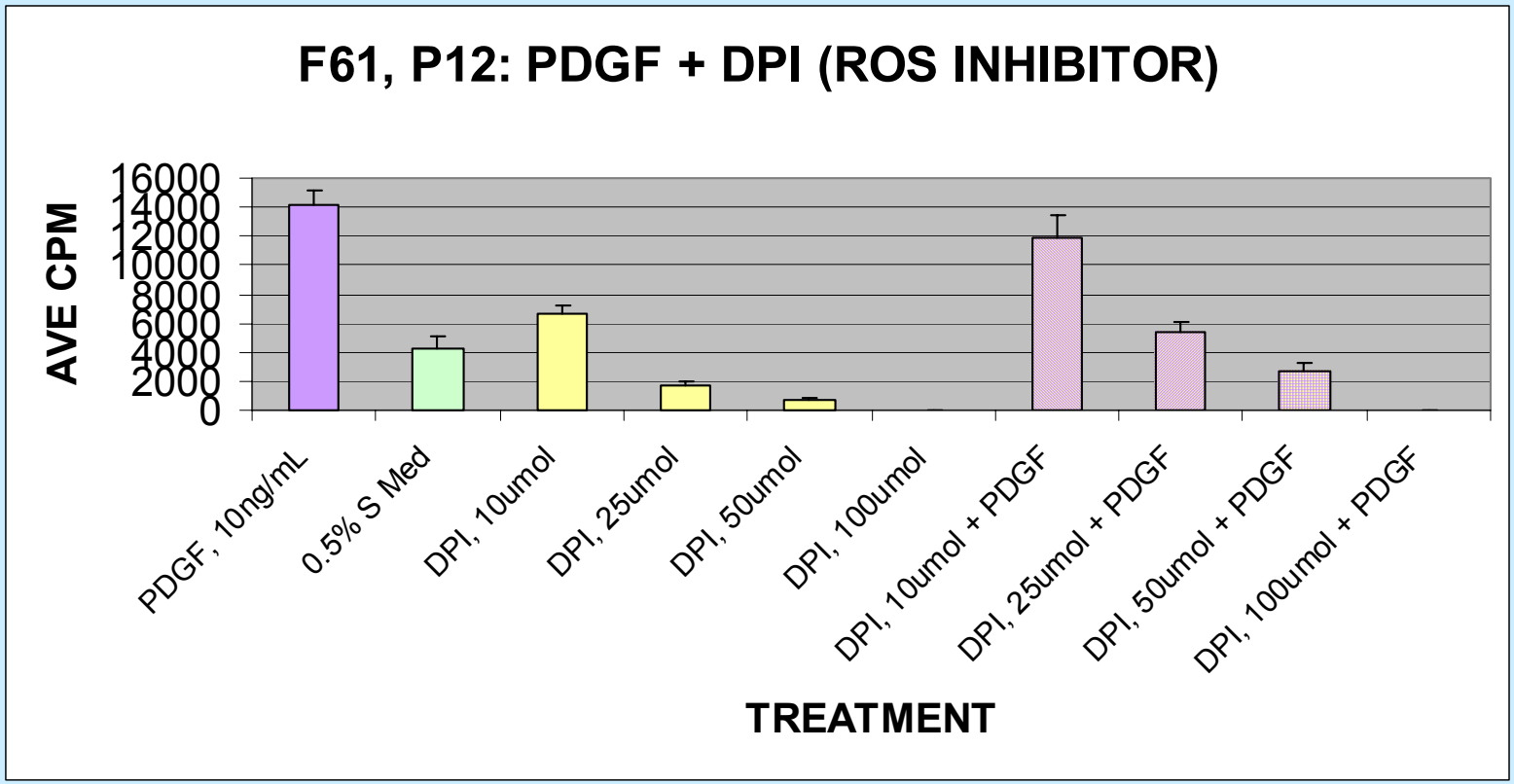


40x, Ph1, Inverted scope, fluorescent light

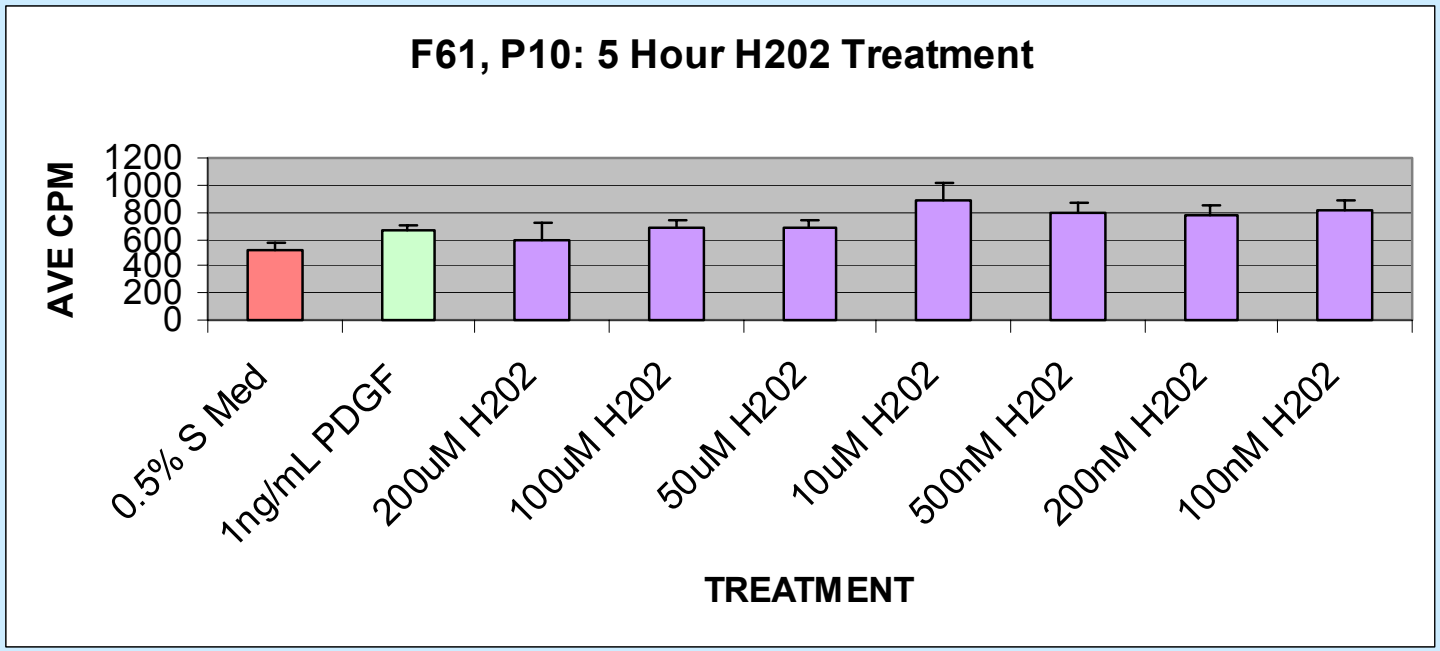


40x, Inverted scope, bright field

Effect of ROS Inhibitor on PDGF stimulated DNA synthesis



Effect of Exogenous H2O2 on DNA Synthesis by Leiomyoma Cells



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(Hypoxia, bacteria)

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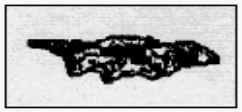

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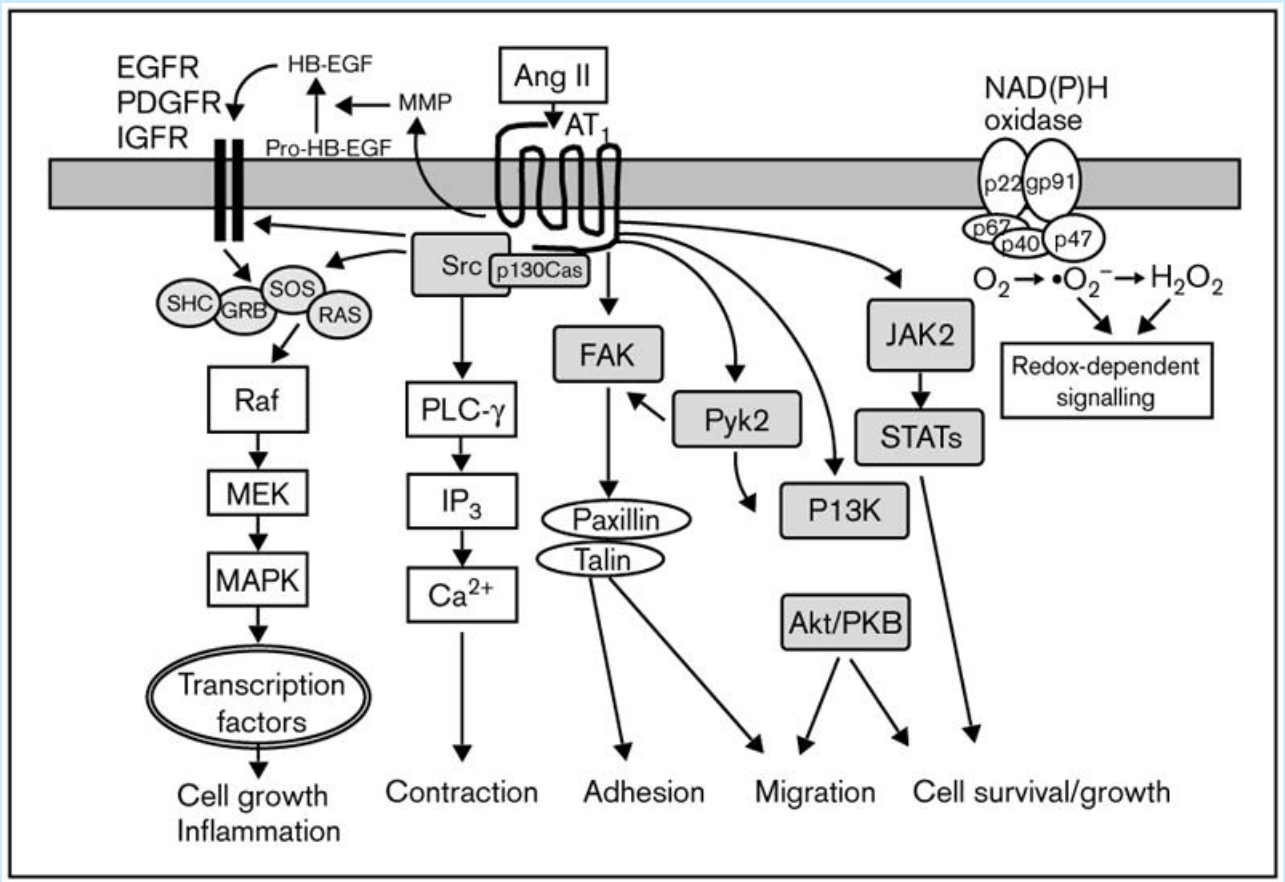
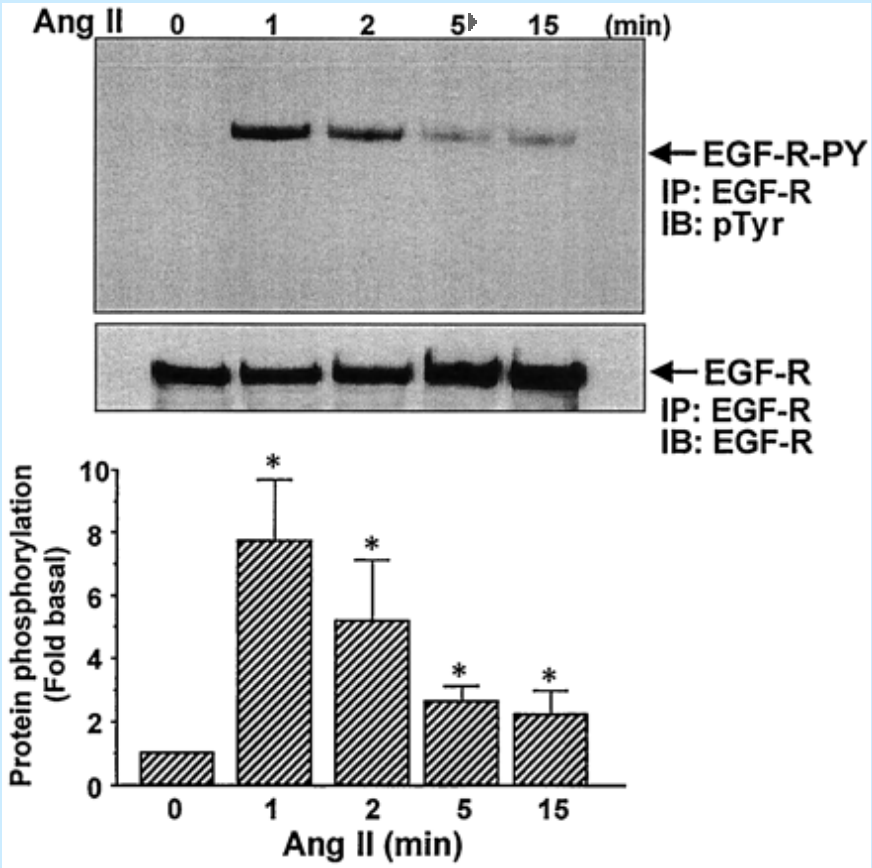
Myoma

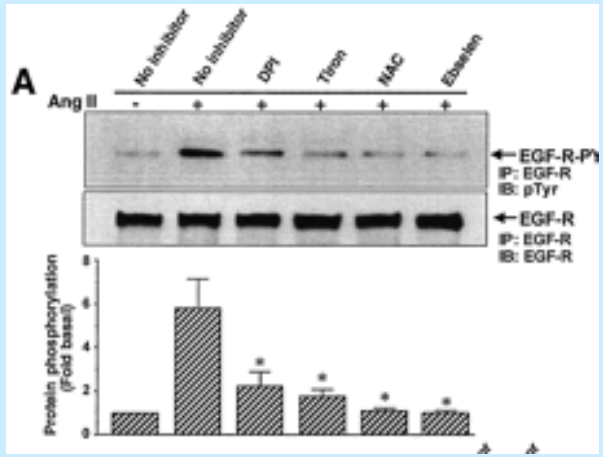
Angiotensin II Mediated Signaling Through Tyrosine Kinases



Touyz, 2003

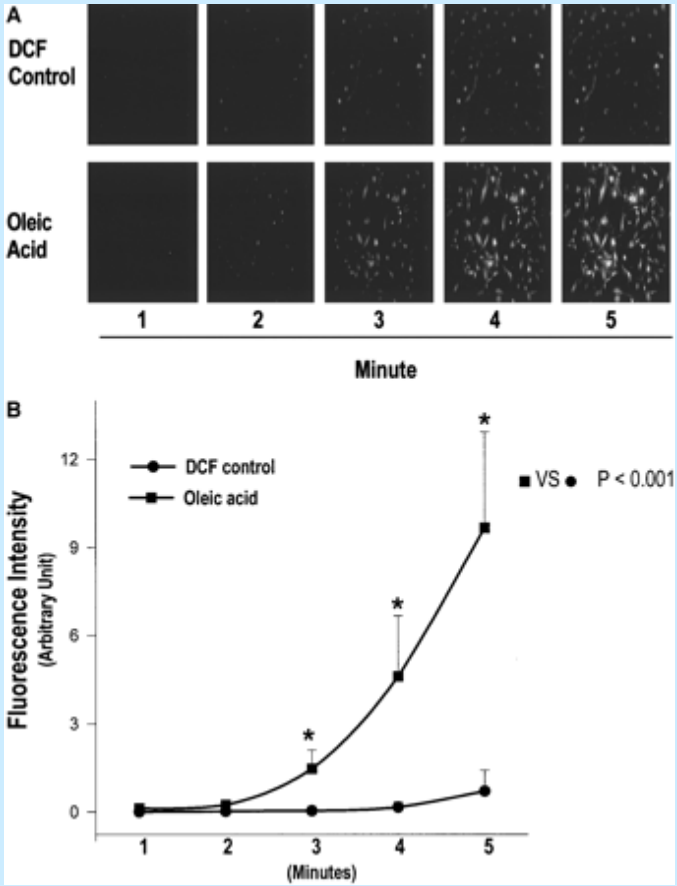
Angiotensin II Induces EGF-R Phosphorylation in Vascular Smooth Muscle Cells

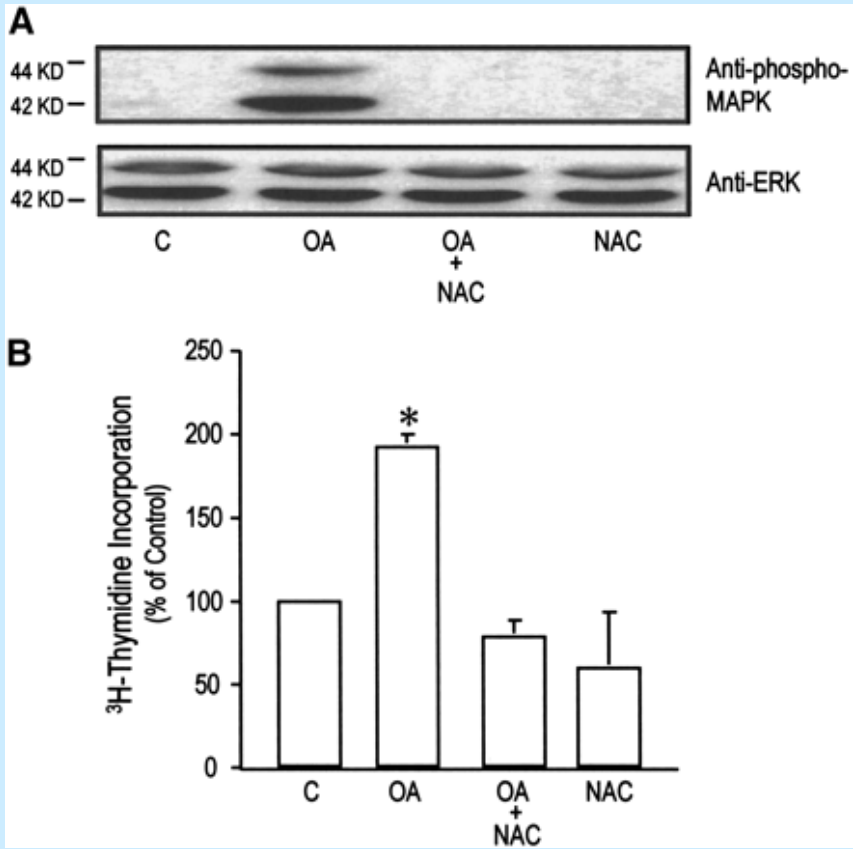




Ushio-Fukai et al., 2001

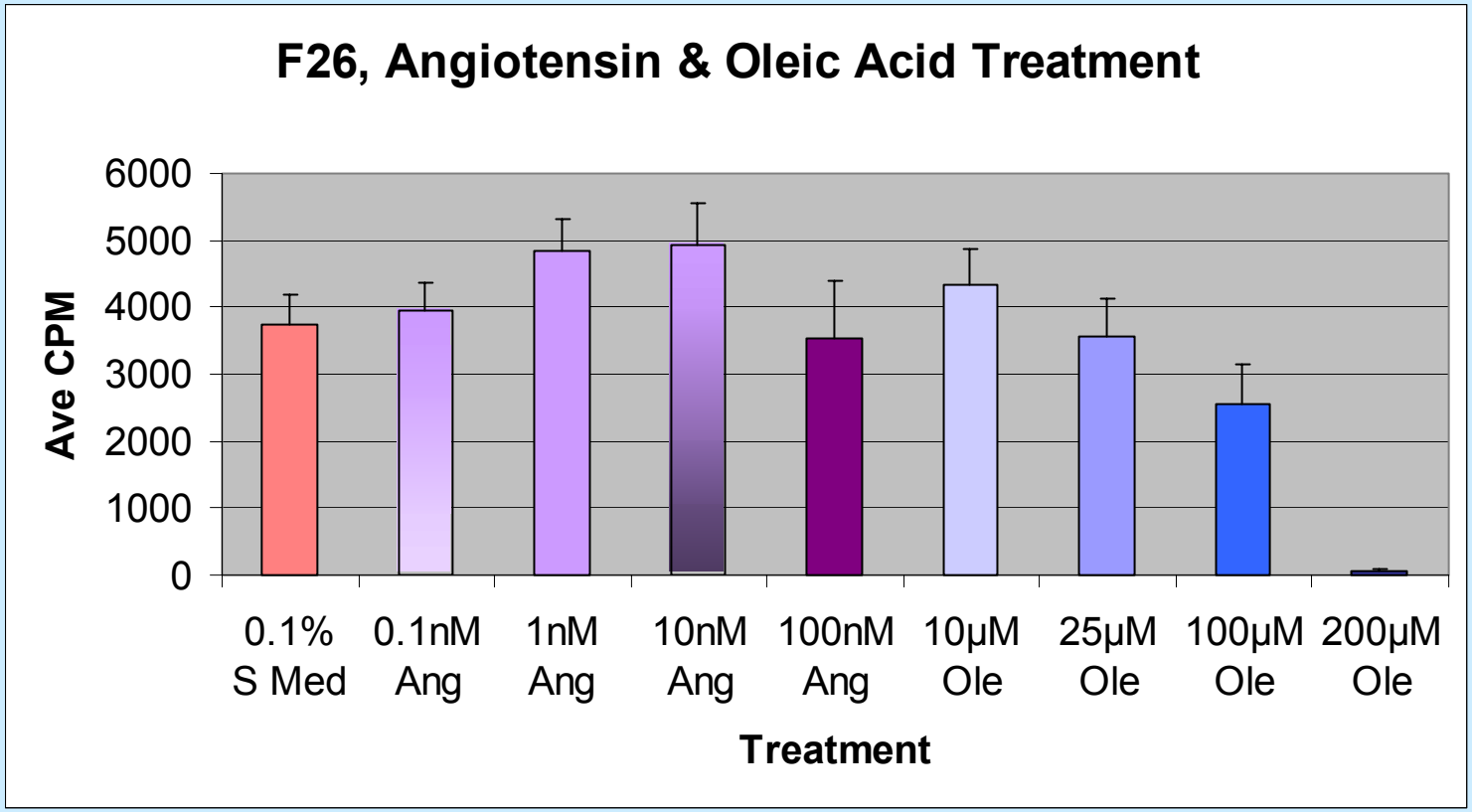
Effect of Oleic Acid on ROS Production and Thymidine Incorporation of Vascular Smooth Muscle Cells



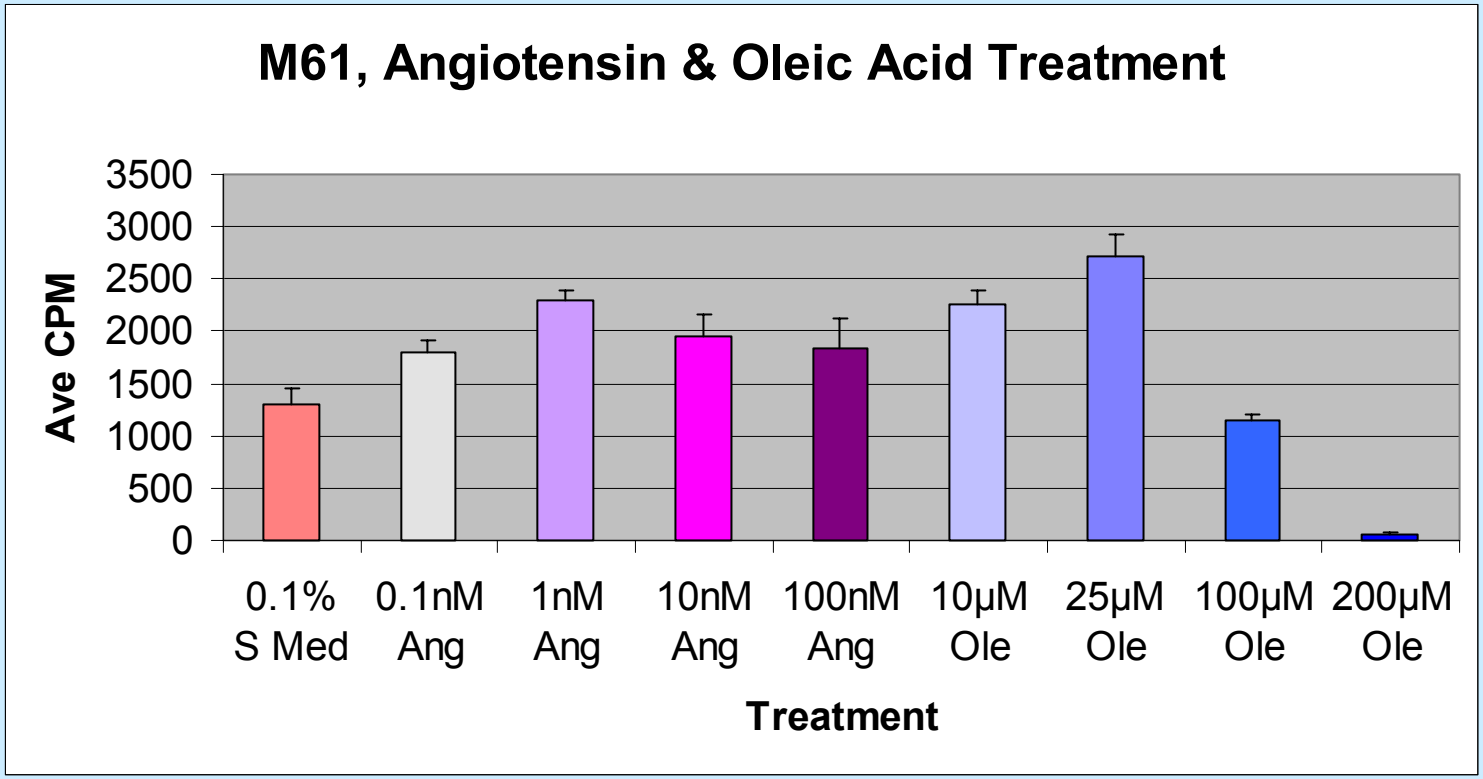


Lu et al., 1998

Effect of Angiotensin and Oleic Acid on Proliferation of Uterine Smooth Muscle Cells

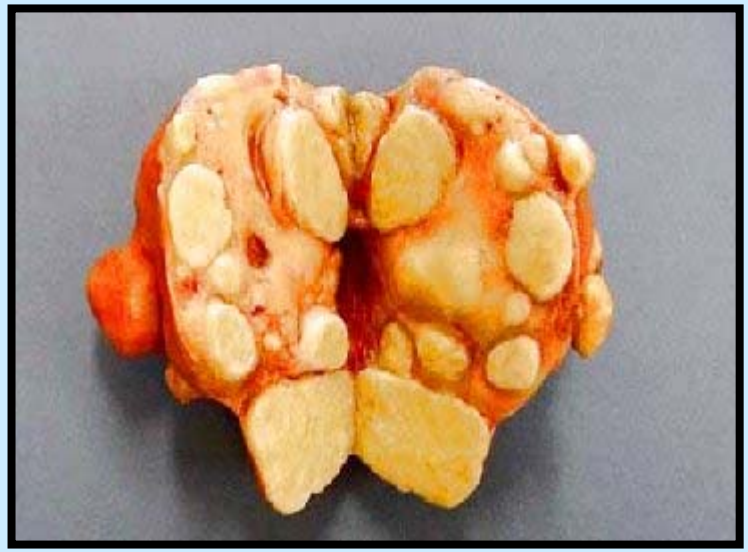


Effect of Angiotensin and Oleic Acid on Uterine SMC Proliferation



What we've learned so far…

- EGF and PDGF increase ROS production by uterine smooth muscle cells, as seen in fluorescent dye experiments
- Other factors such as angiotensin II and oleic acid may also regulate proliferation of uterine smooth muscle cells.



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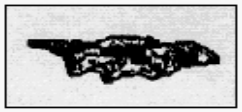

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Myoma

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