

Approaches for Characterizing Activities and Constituents of Complementary/Alternative Medicine Therapies

PC-SPES as a paradigm

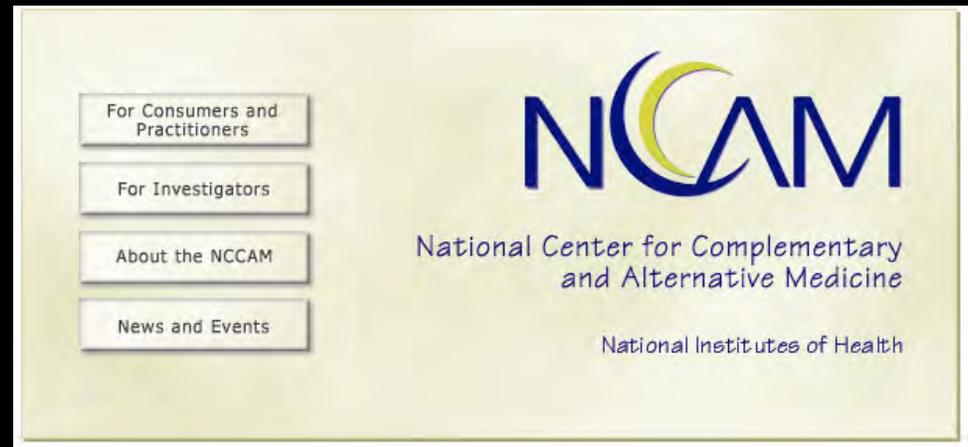
Peter Nelson

Fred Hutchinson Cancer Research Center

Complementary/ Alternative Medical Therapy (CAM)

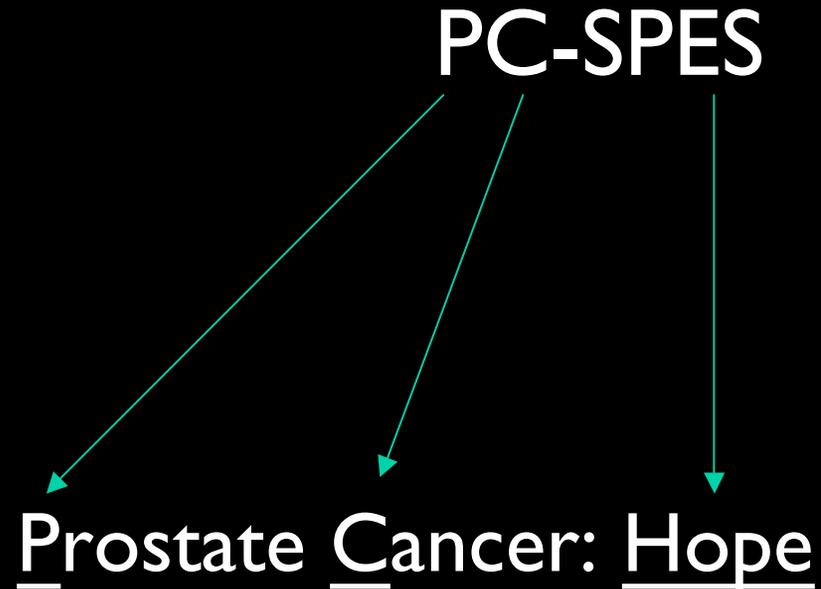
- Major form of treatment and health maintenance
- 42% of the US population uses some form of CAM
- 23 Billion dollars spent annually
- 629 million visits/year to CAM providers
- Wide variety of therapies:
 acupuncture/massage/herbals....

- Little knowledge of:
 - safety
 - efficacy
 - drug interactions
 - mechanisms of action



27-37% of prostate cancer patients use CAM

PC-SPES



Prostate Cancer: Hope

The diagram consists of the text 'PC-SPES' at the top center. Three red arrows originate from the letters 'P', 'C', and 'S' respectively, pointing downwards to the underlined letters 'P', 'C', and 'H' in the text 'Prostate Cancer: Hope' below.

“It Works Like Hormone Therapies, Only Better”

Julian Whitaker, M.D. Whitaker Wellness Institute

PC-SPES

Mixture of 8 natural herbs (7 China/1 USA):



Isatis indigotica

glycyrrhiza glabra

panax pseudo ginseng

ganoderma iucidum karst

dendranthera morifolium

Isatis indigotica

Robdosia rubescens

Scutellaria baicalensis

- Manufactured by Botanicalab (Brea, CA)
- Sold as a dietary supplement
- Cost: \$200-400/month (dose of 9 320 mg capsules per day)

'Active' Ingredients in PC-SPES

Serenoa repens

phytoestrogen

glycyrrhiza glabra

antimutagenic/inhibition of steroid dehydrogenase

panax pseudo ginseng

lowers cancer incidence/antiproliferative

ganoderma iucidum

glycans/antisarcoma activity

dendrantherma morifolium

Isatis indigotica

antineoplastic/immunostimulatory activity

Robdosia rubescens

Scutellaria baicalensis

flavanoid/antiproliferative and lipoxygenase
inhibitory activity/DNA topoll inhibition/apoptosis
induction/

Stressed by manufacturer that it is the combination of herbs, rather than each of them individually, which is responsible for the for the overall *in vivo* antineoplastic activity of the preparation.

PC-SPES: Clinical Results

Prospective Trial of the Herbal Supplement PC-SPES in Patients with Progressive Prostate Cancer.

Small et al (2000) JCO 18:3595-3603.

- 70 men enrolled, 61 evaluable
- 34 hormone refractory
- 27 hormone naïve

- 9 PC-SPES capsules per day
- Followed PSA

- >50% PSA reduction in 27/27 hormone naïve
- >50% PSA reduction in 19/34 castration resistant

PC-SPEs: Side-Effects

Gynecomastia	70%
Nausea (mild)	12%
Diarrhea (mild)	33%
Loss of libido/potency	60%
Thrombosis	~3%
Hemorrhage	case report*
Expense	100%

Clinical and Biologic Activity of PC-SPES in Prostate Cancer

Summary:

- PC SPES has potent estrogenic activity *in vitro*
- PC-SPES has cytotoxic activity *in vitro*
- Lowered PSA and testosterone levels *in vivo*
- Multiple phytoestrogens (in components of PC-SPES)
- Side-effect profile (in patients) similar to estrogens
- Suggests clinical activity due at least in part
to estrogenic activity....
- DES??

Cautions re: uncontrolled use...[editorial NEJM (1988) 17;339]

What is the molecular basis for PC-SPES activity?

➤ Is PC-SPES simply acting on known pathways?

(...that can be modulated using known drugs: e.g. **DES**?)

-Controlled Production

-FDA oversight

-Cost?

➤ Can activity be attributed to specific component(s)?

What is the molecular basis for PC-SPES activity?

➤ Is PC-SPES simply acting on known pathways?

(...that can be modulated using known drugs: e.g. **DES?**)

-Controlled Production

-FDA oversight

-Cost?

➤ Can activity be attributed to specific component(s)?

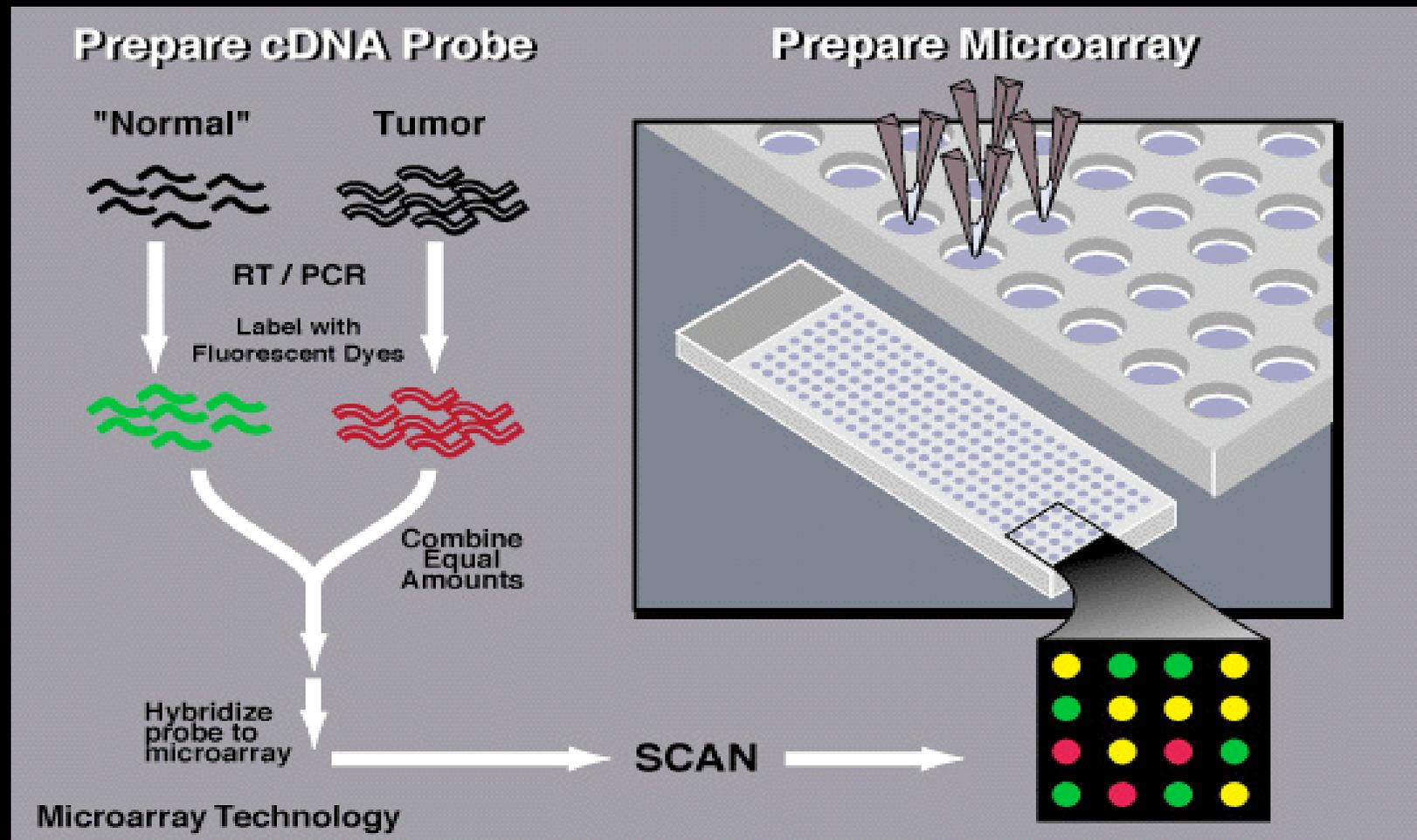
Strategy: Use a gene expression profile determine:

-Mechanisms/pathways of activity

-Active components

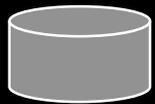
-Lot to lot variation?

cDNA Microarray

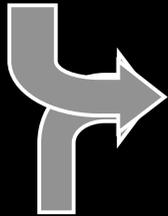


Identification of PC-SPES-regulated genes in cancerous prostate epithelial cells

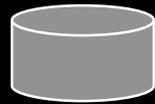
LNCaP + PC



mRNA ●



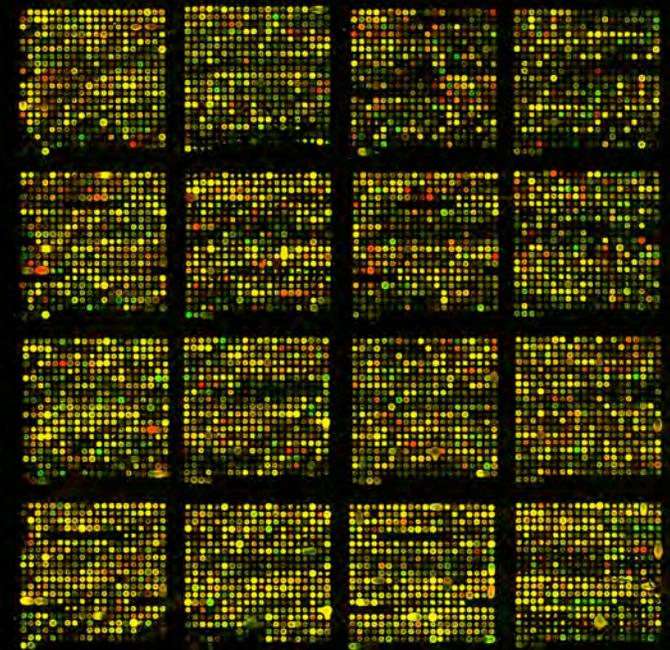
LNCaP - PC



mRNA ●

LNCaP cells grown for 24 and 48 hours in media supplemented with PC-SPES--RNA harvested and labeled.

LNCaP cells grown for 24 and 48 hours--RNA harvested and labeled (vehicle control)

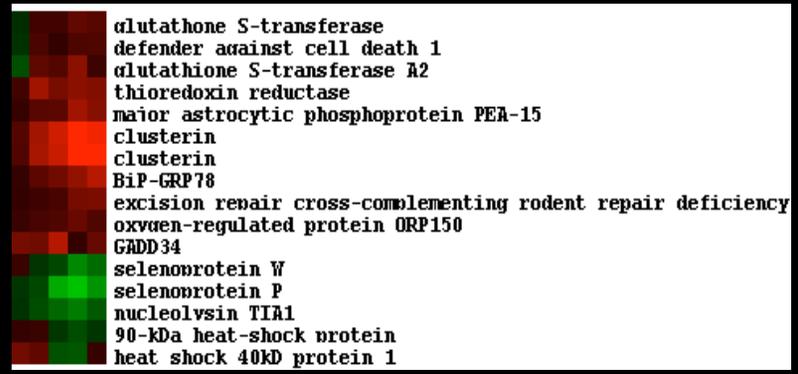


TIME →

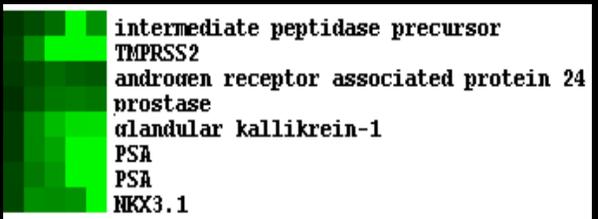
cytoskeleton



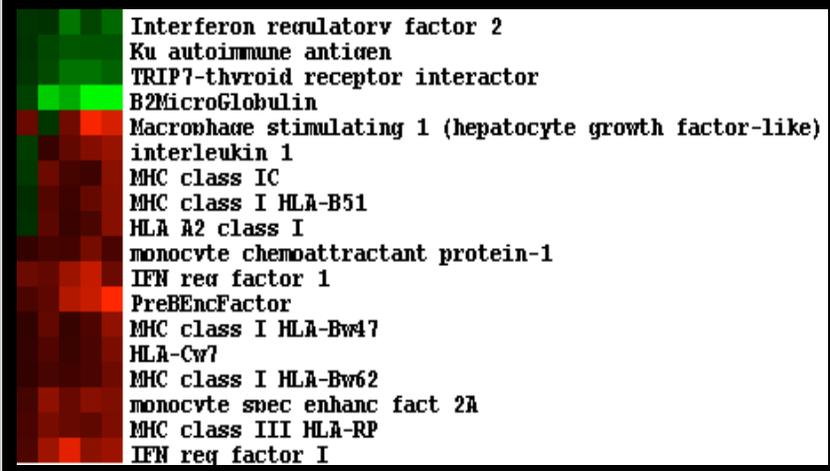
apoptosis/ cell stress



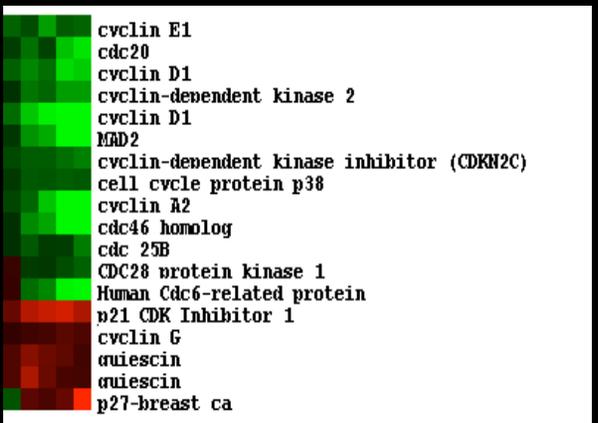
androgen



immune modulation



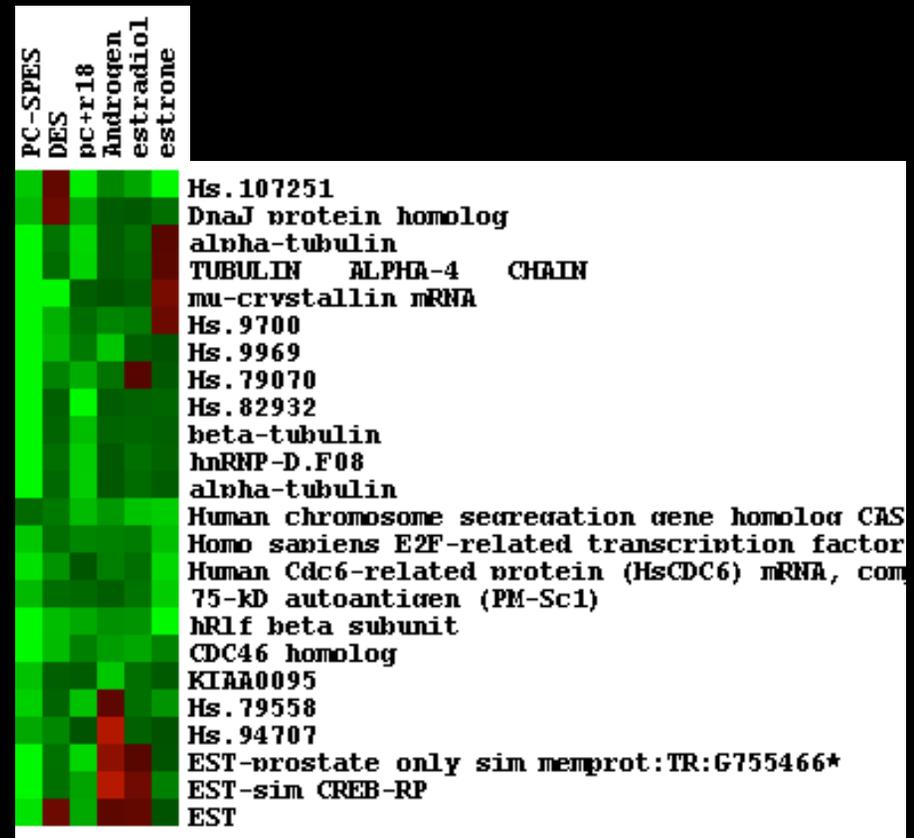
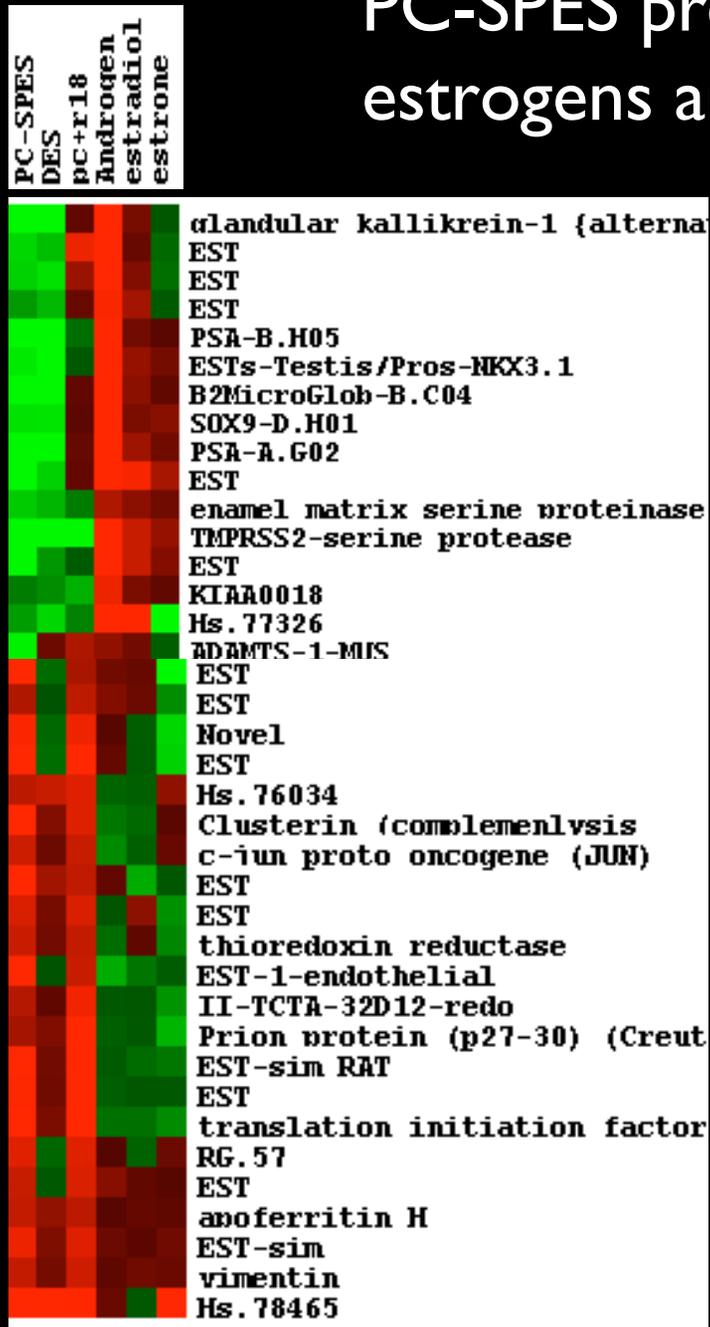
cell cycle



fold change

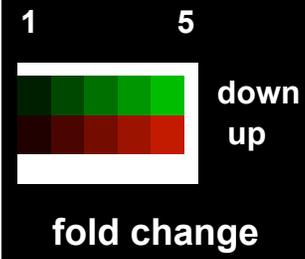
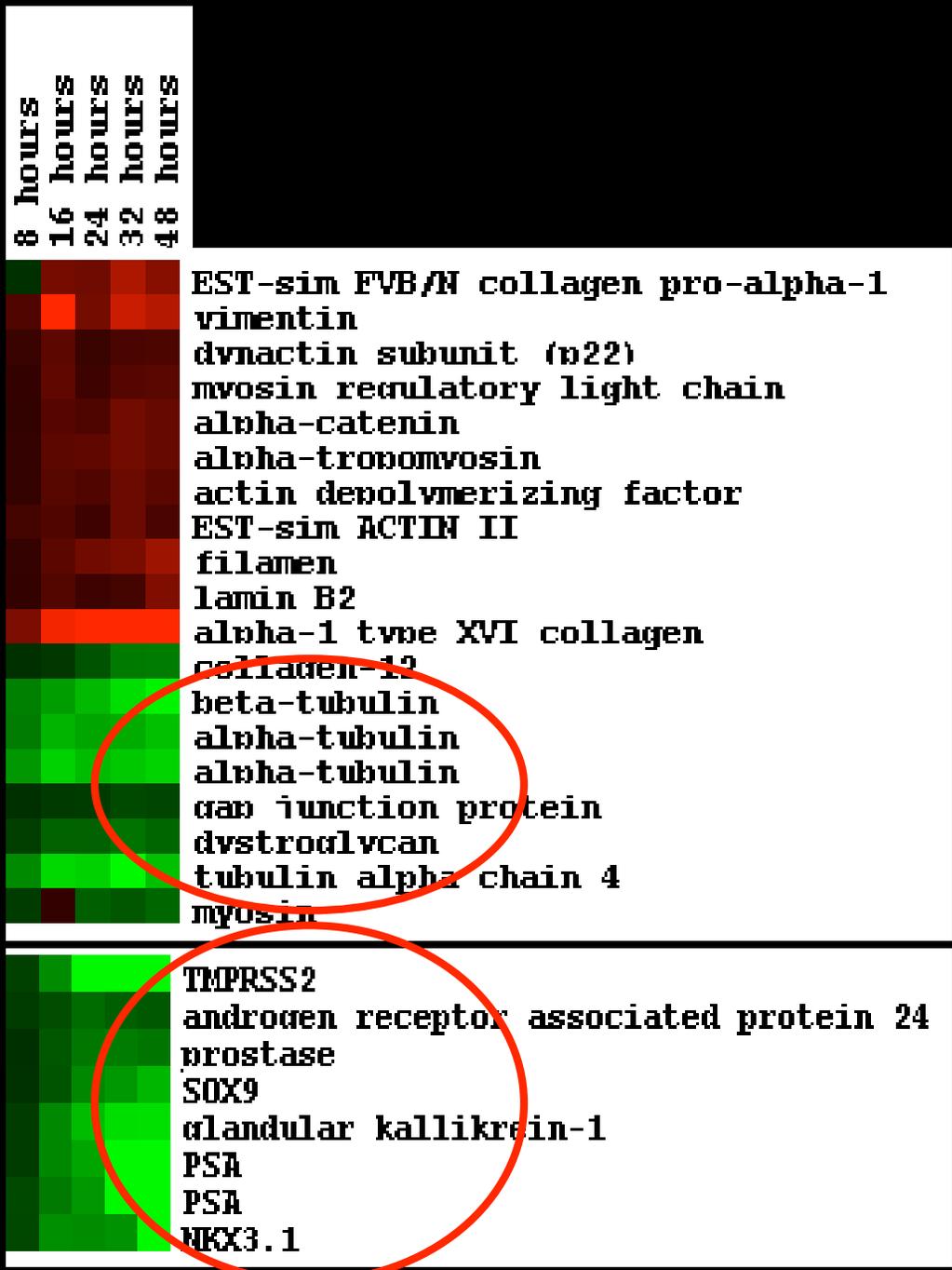


PC-SPES profile distinct from known estrogens and androgen



Androgen
Regulated

Cytoskeleton



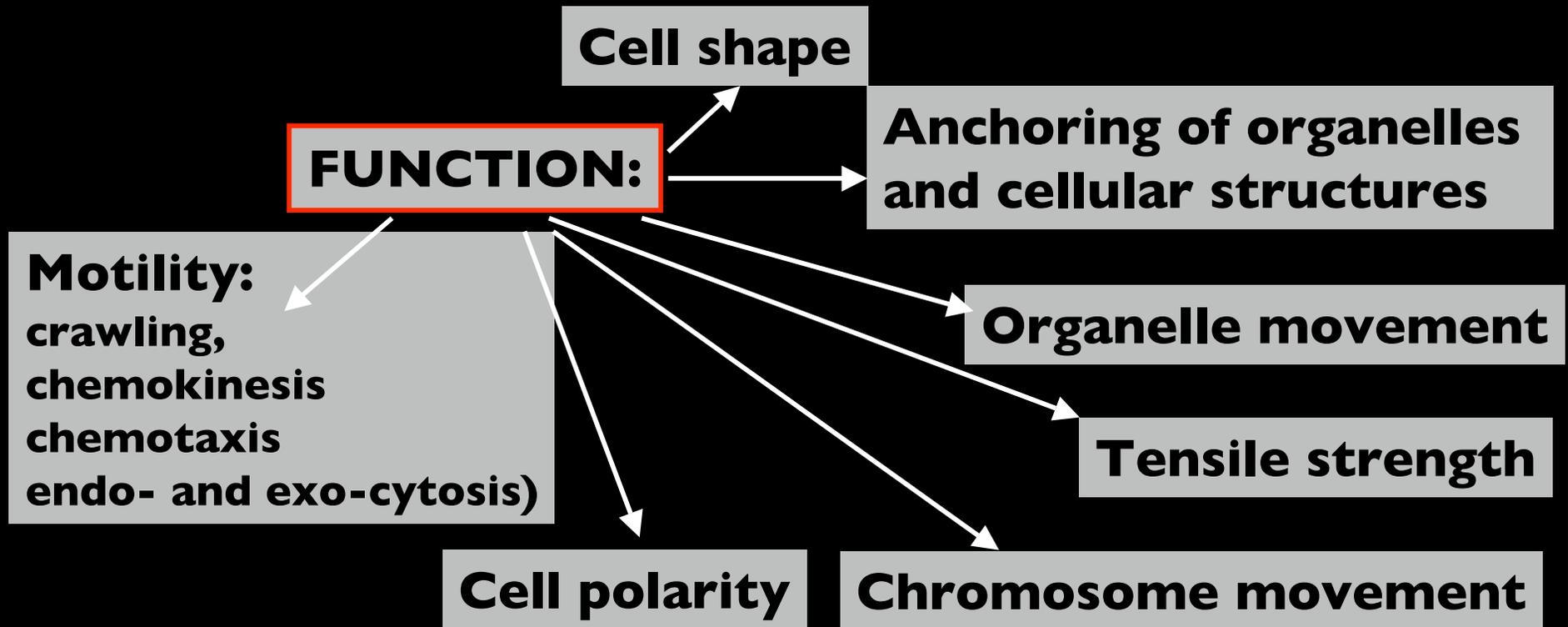
PC-SPES and the CYTOSKELETON

Cytoskeleton: complex network of filamentous proteins extending throughout the cytoplasm

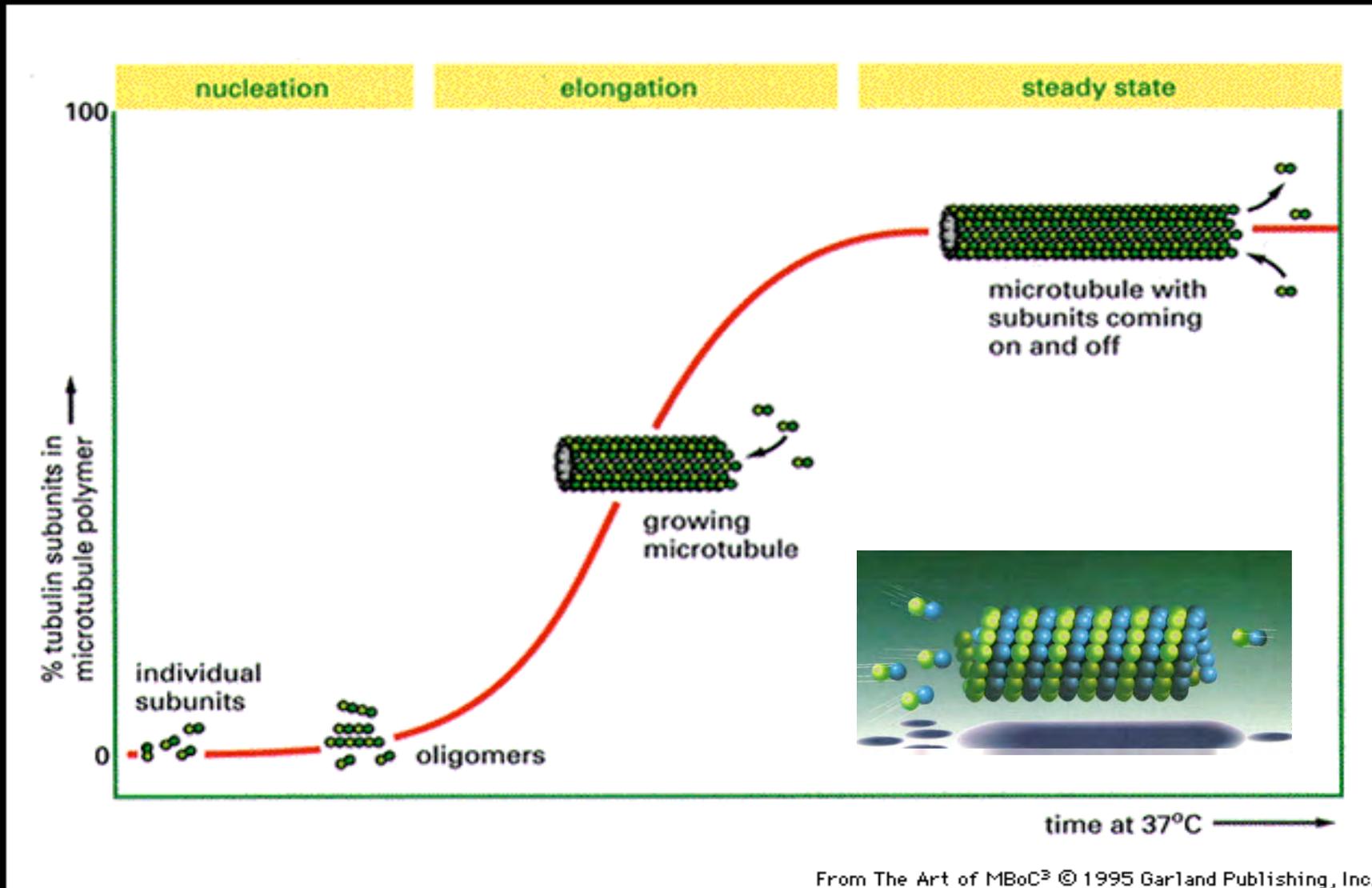
Three types of filaments: Microtubules

Microfilaments

Intermediate Filaments



Tubulin polymerization



Drugs Targeting Microtubules

Target the labile mitotic spindle >> antimitotic drugs<<
Preferentially kill abnormally dividing cells

Colchicine (alkaloid from meadow saffron)

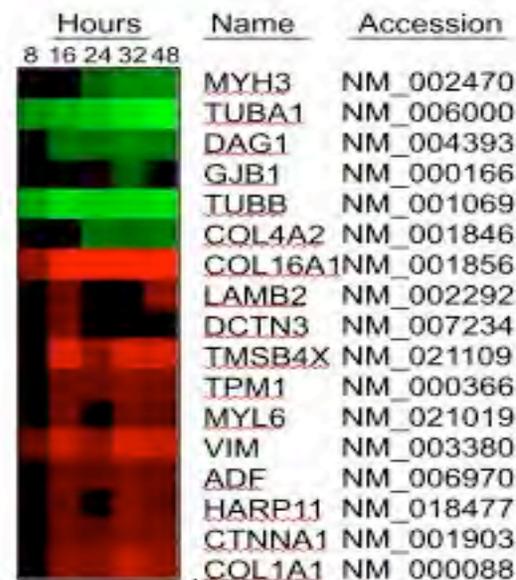
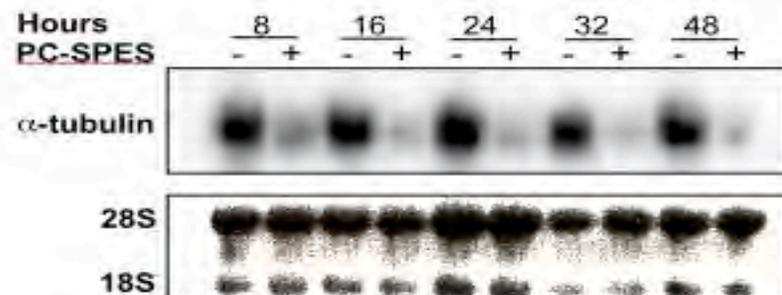
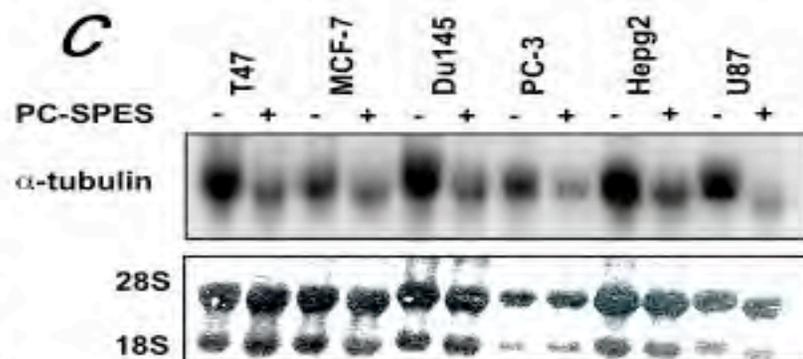
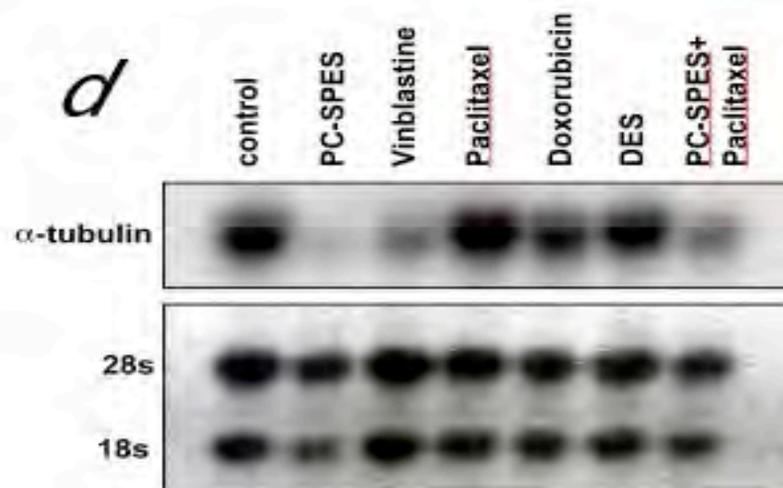
- binds irreversibly to β -tubulin monomer
- prevents polymerization, destabilizes polymers,
(ancient treatment for gout)

Taxol* (derived from bark of yew trees)

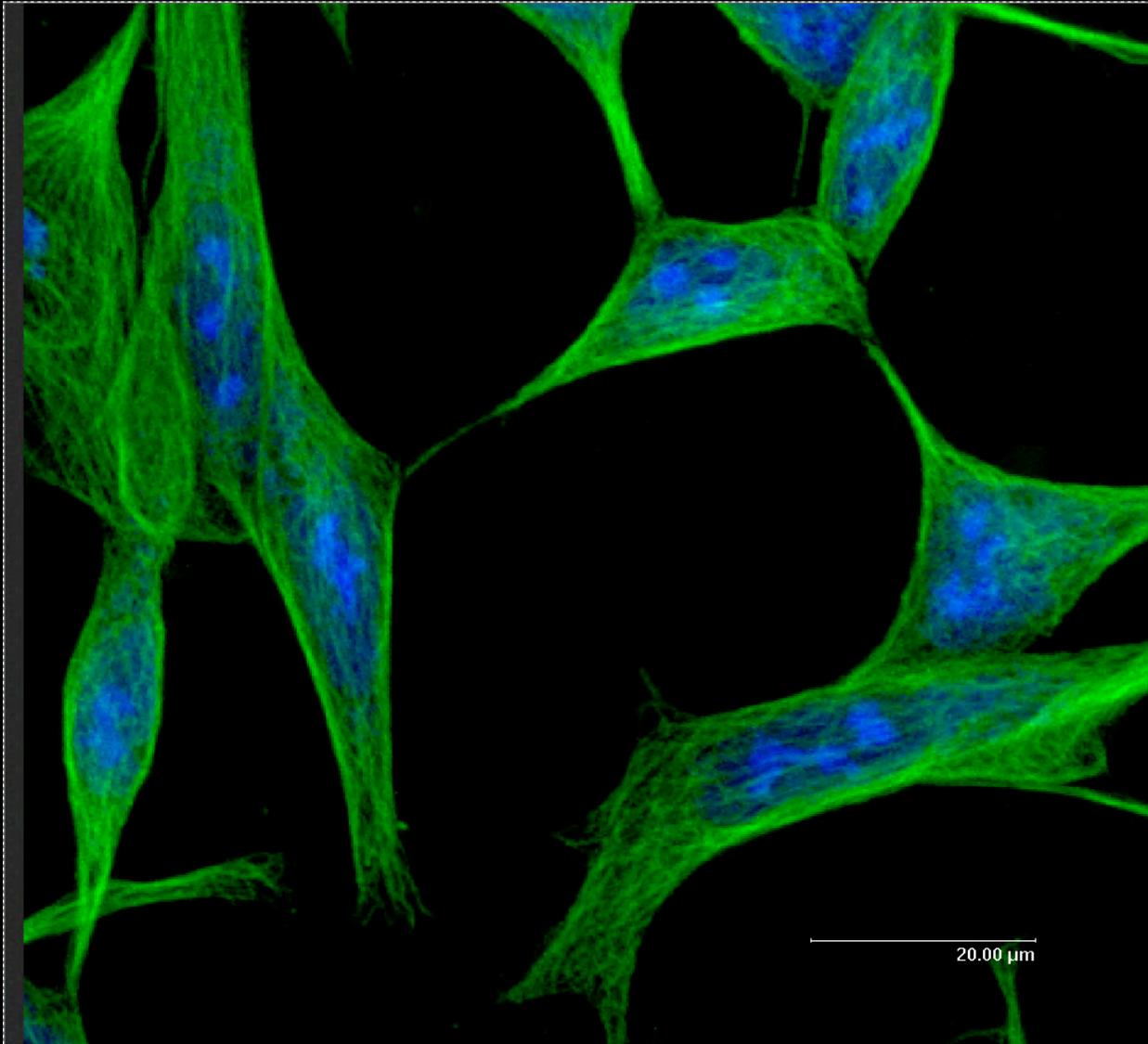
- binds to MT: stabilization (prevent depolymerization)
- cell cycle arrest

Vinblastine*, Vincristine*, Nocodazole

- bind to tubulin monomers,
- prevent polymerization of tubulin
- cell cycle arrest

a**b****c****d**

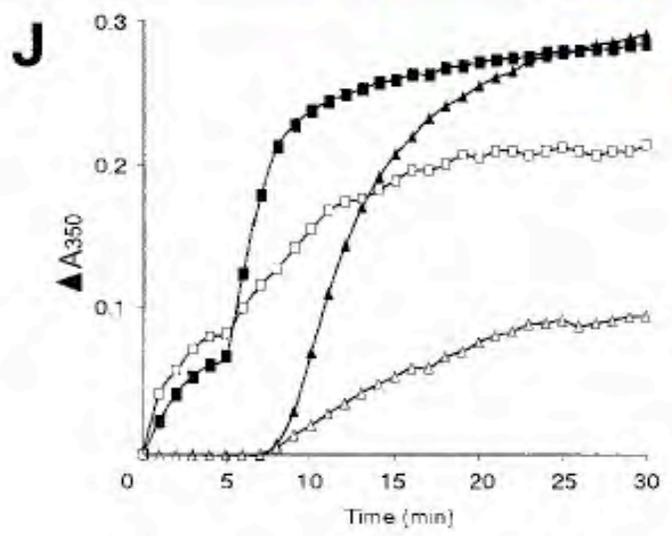
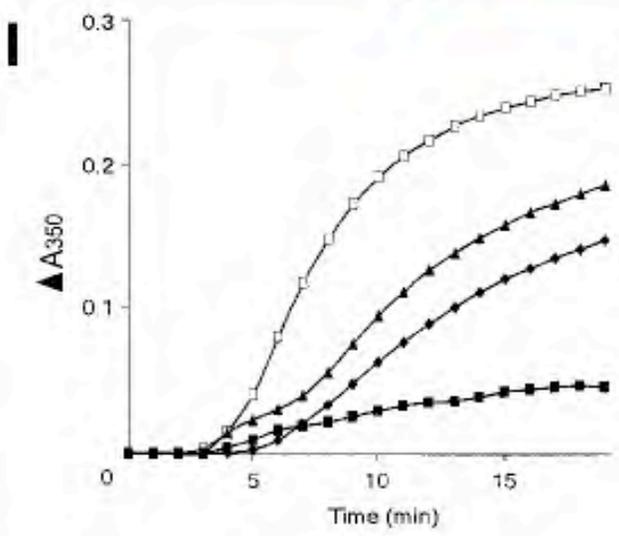
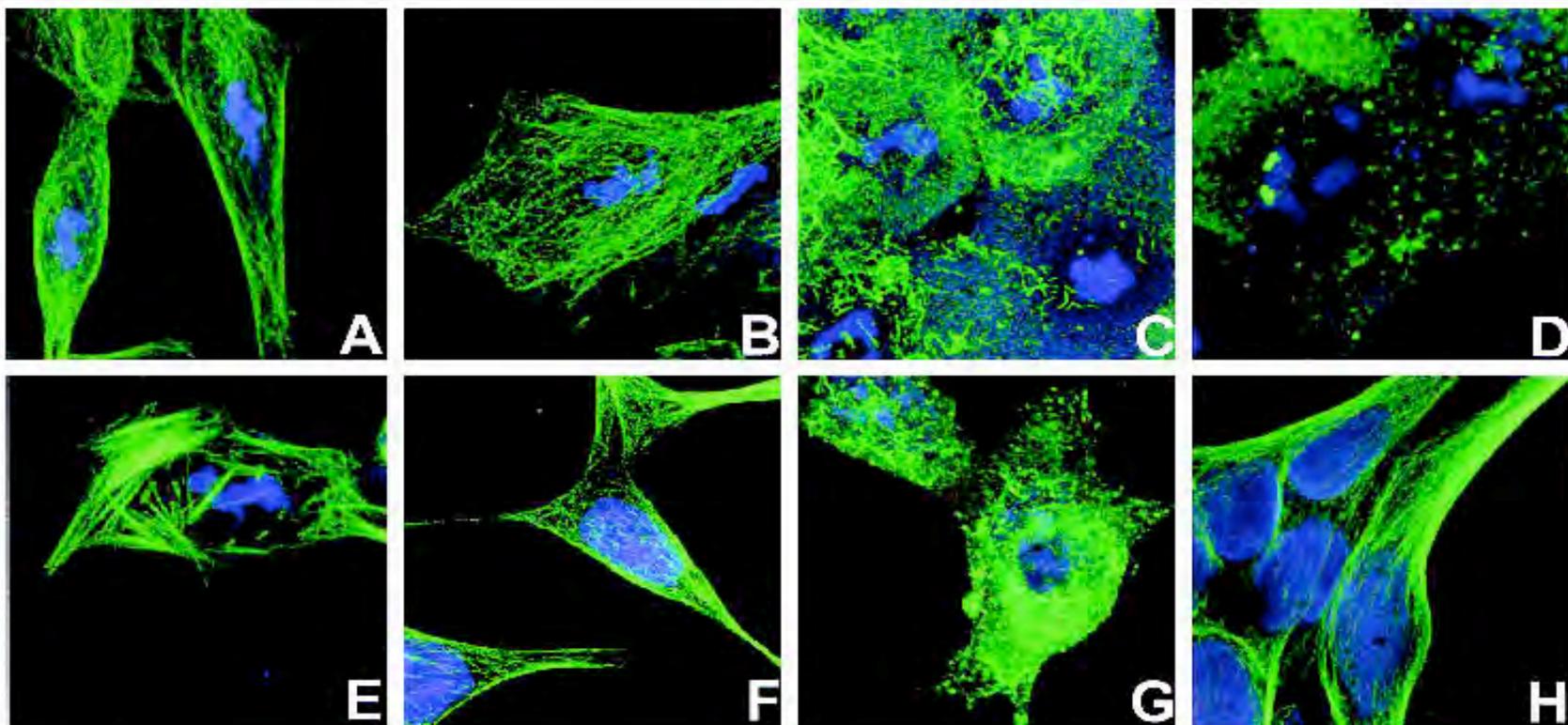
Tubulin immunofluorescence



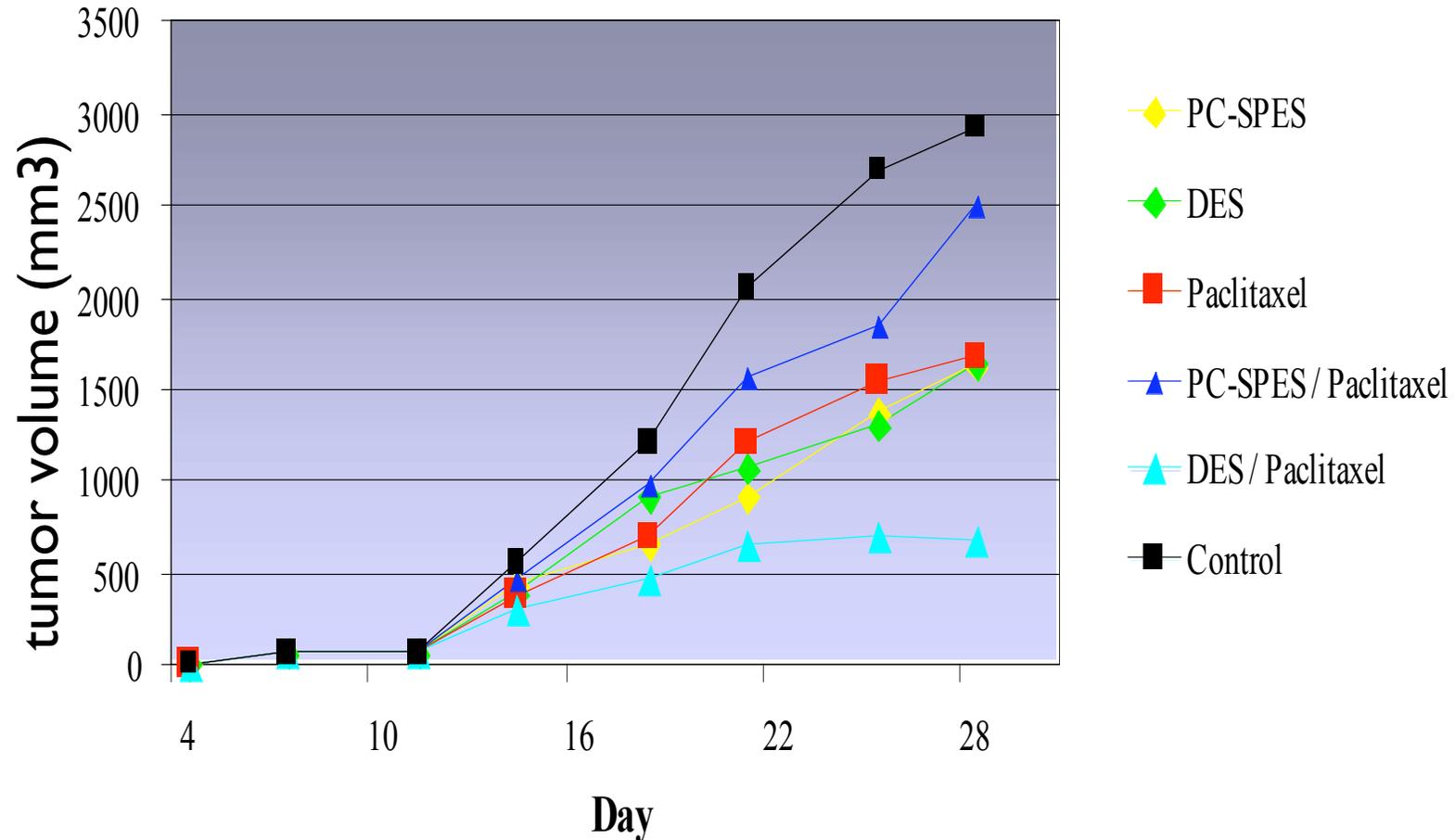
Control

Anti-alpha-tubulin
Alexa 488 (Green)

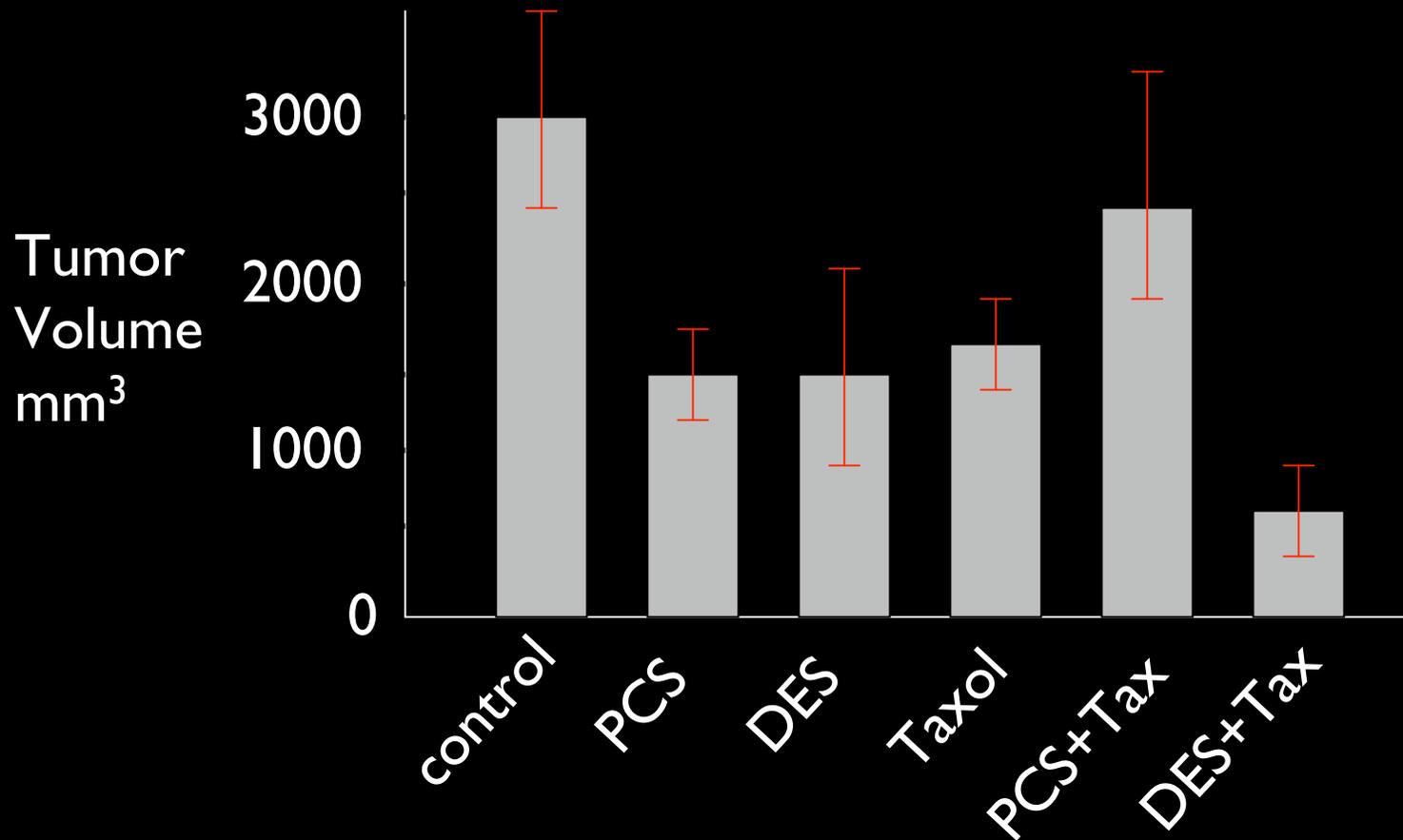
Chromatin
Toto-3 (Blue)



Mouse prostate xenograft study: AIPC



Prostate Xenograft Volume at 28 days



PC-SPES Separation and Purification

Ethanol solubilize dry herbs



Methylene chloride extraction



HPLC- normal phase, collect fractions



Test HPLC fractions for activity

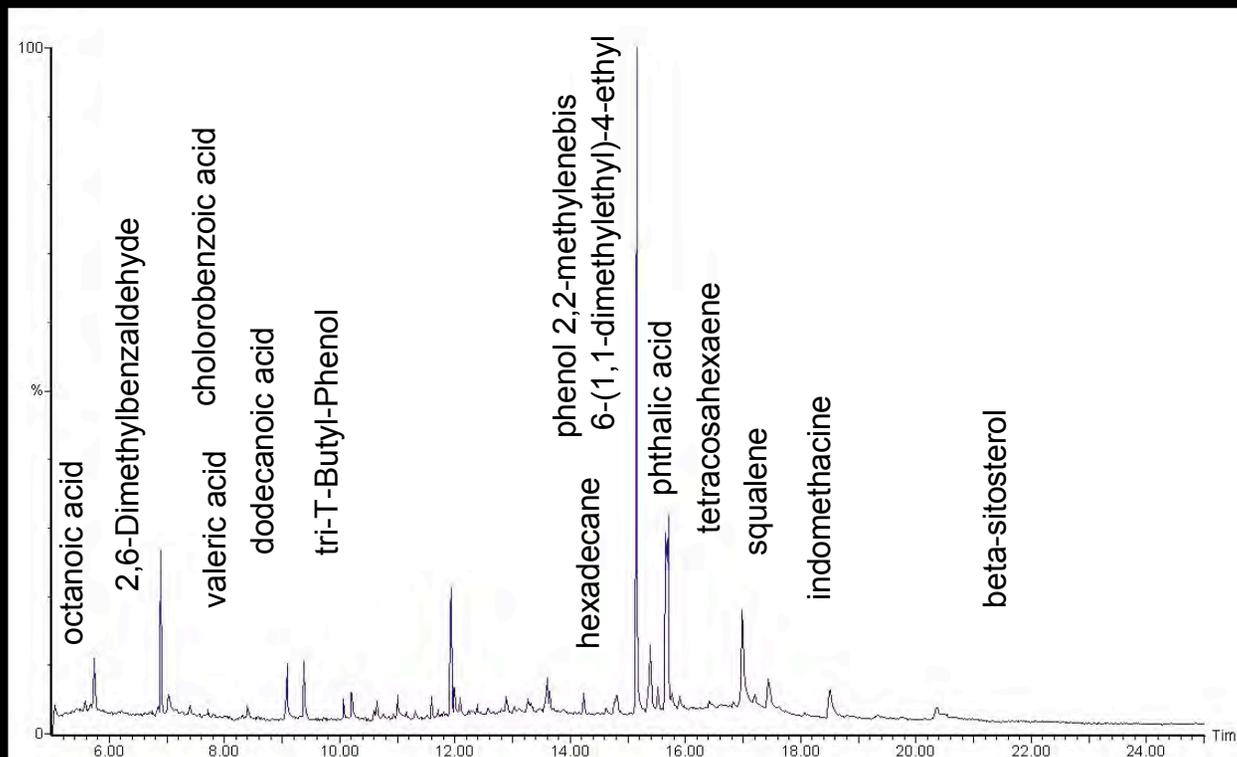


GC Mass spec.- Identify compounds in active fraction

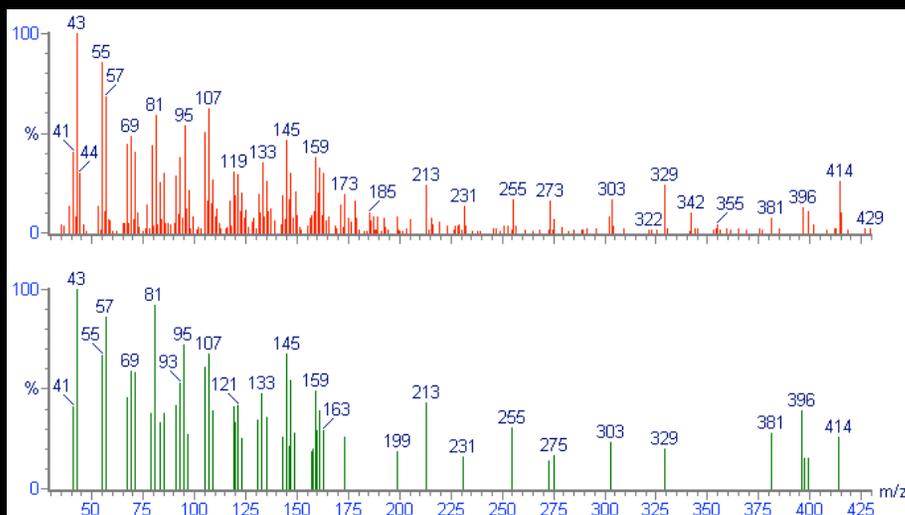


NMR- detect structures of compounds

A.



B.



Stigmast-5-en-3-ol beta CAS# 83-47-5
C₂₉H₅₀O MW=414 F=86.8 R= 91.3

PC-SPES fraction 16
Compounds with greater than 70% match

dimethylbenzaldehyde

chlorobenzoic acid

valeric acid

dodecanoic acid

phthalic acid

indomethacine ←

beta-sitosterol

octanoic acid

decanoic acid

propenoic acid

tetracosahexaene

squalene

phenol 2,2-methylenebis

ARTICLE

Herbal Composition PC-SPES for Management of Prostate Cancer: Identification of Active Principles

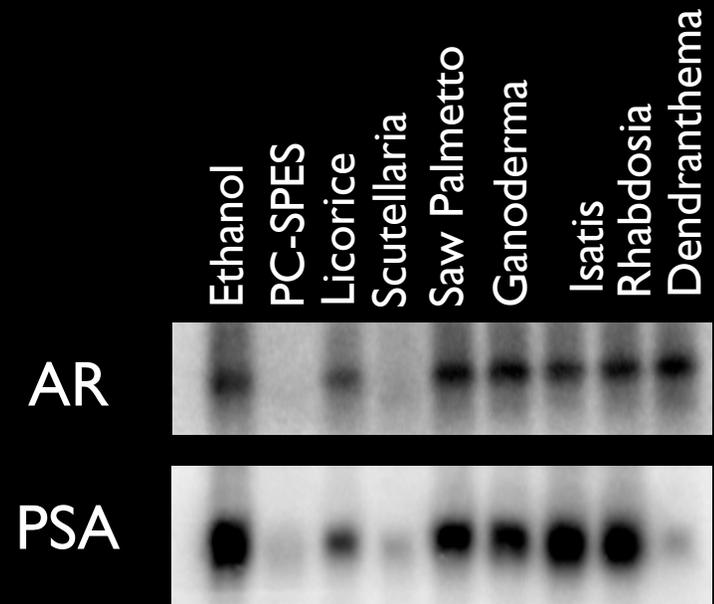
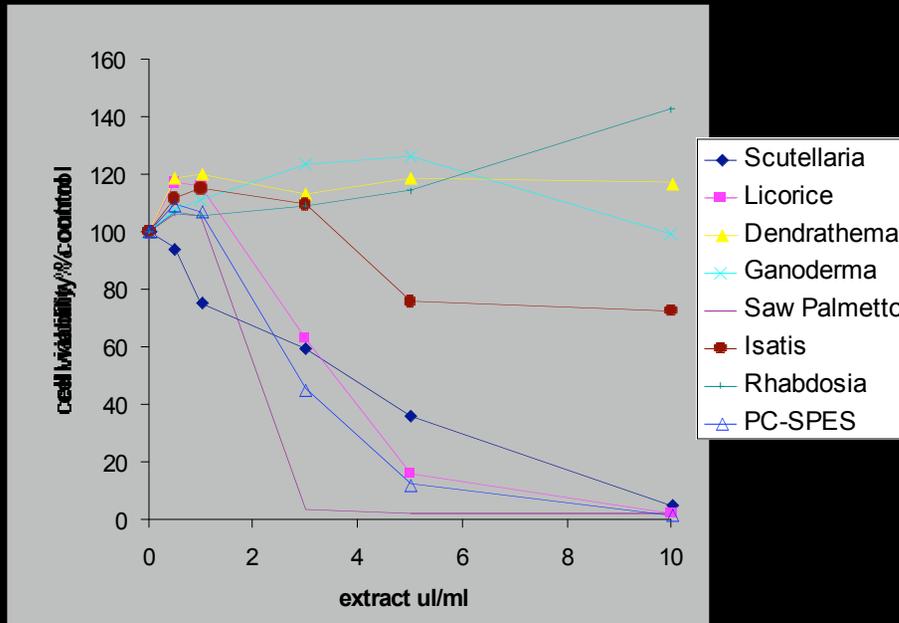
Milos Sovak, Allen L. Seligson, Martin Konas, Marian Hajduch, Marek Dolezal, Miroslav Machala, Robert Nagourney

Table 2. Concentration of indomethacin (IN), diethylstilbestrol (DES), warfarin (WA), licochalcone A (LA), and baicalin (B) in different PC-SPES lots*

Lot No. (manufacture date)	IN, mg/g	DES, µg/g	WA, µg/g	LA, µg/g	B, mg/g
5436285 (10/1996)	1.07	122.35	n/d	48.5	n/d
5438126 (06/1998)	13.19	114.74	n/d	12.8	21.2
5438763 (06/1998)	12.24	154.00	n/d	10	7.1
5438196 (07/1998)	12.81	159.27	560	19.0	7.5
5438362 (03/1999)	3.44	107.28	341	3.8	15.0
5430125 (06/2000)	1.56	46.36	527	14.1	12.5
5431106 (04/2001)	0.70	11.92	398	27.6	28.8
5431219 (08/2001)	0.89	n/d	483	289.2	38.8

*Data are the mean (95% confidence intervals). The approximate manufacturing dates of the lots were found to precede the label expiration dates by 2 years. n/d = not detectable.

Examination of 7 Herbs in PC-SPEs



Decrease cell growth:

- Scutellaria
- Saw Palmetto
- Licorice

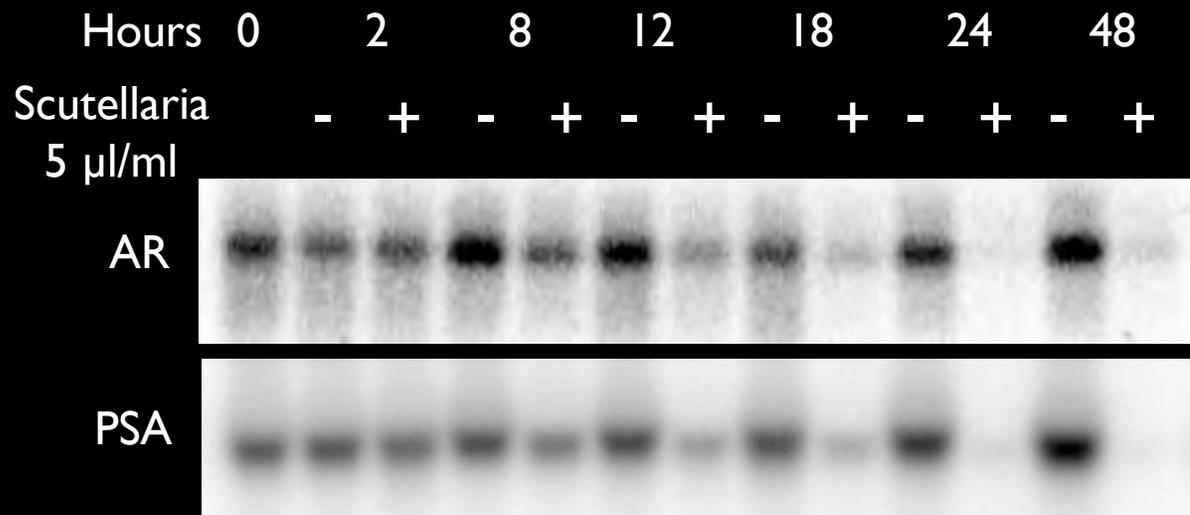
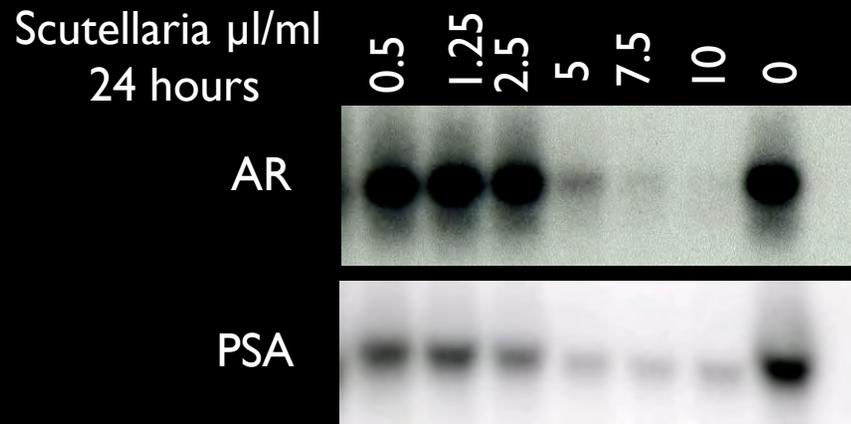
Decrease PSA:

- Licorice
- Scutellaria
- Dendrathema

Decrease AR:

- Scutellaria

Scutellaria Decreases AR expression

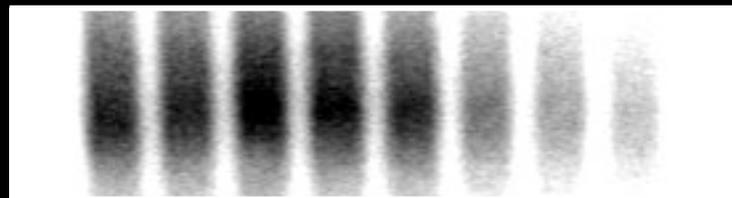


Scutellaria baicalensis

- Used to treat inflammatory disorders in China for centuries
- High flavonoid content (35%)
- antioxidant/inhibition of TNF, NO, COX-2...
- Predominant flavonoids- baicalein 28%
wogonin 10%

baicalein μM 0 0.5 1.0 5.0 10 20 30 50

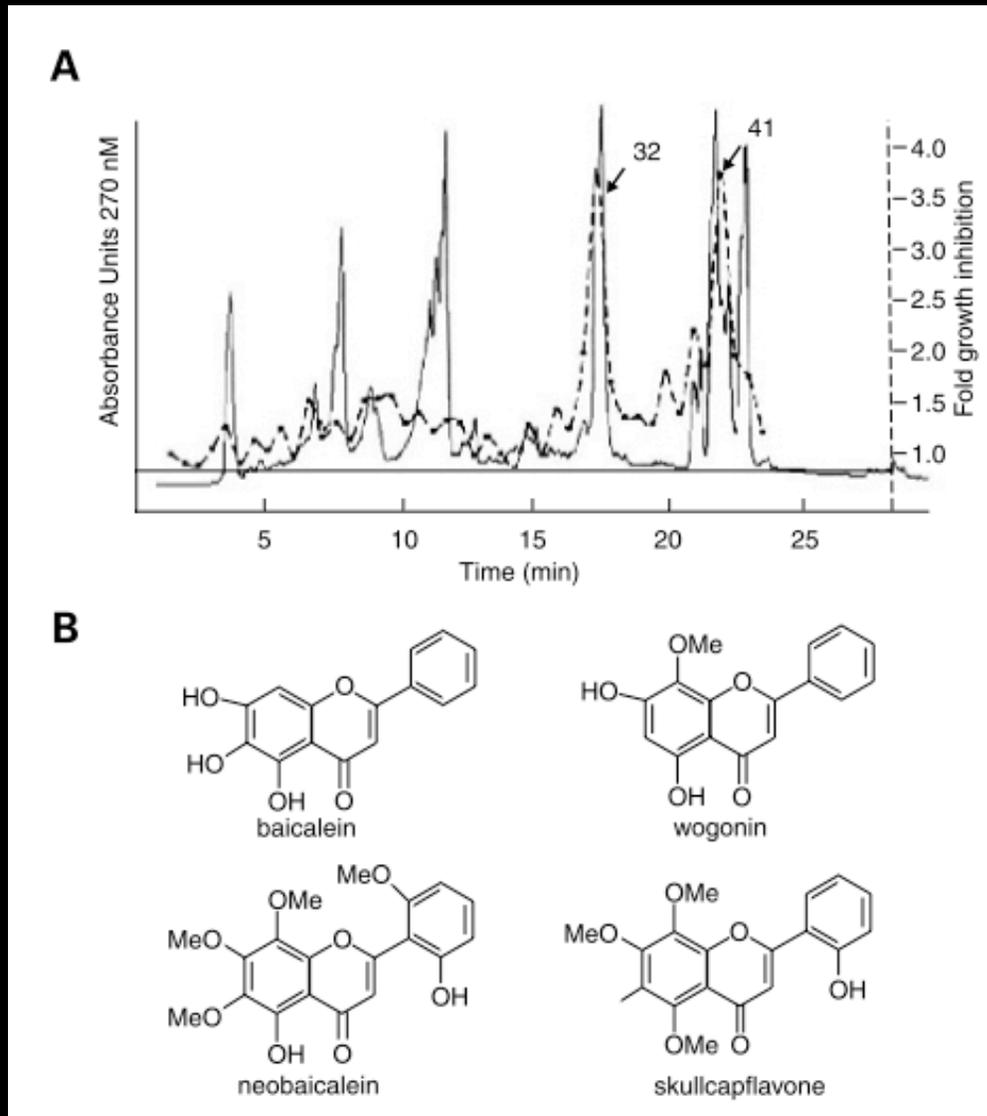
AR



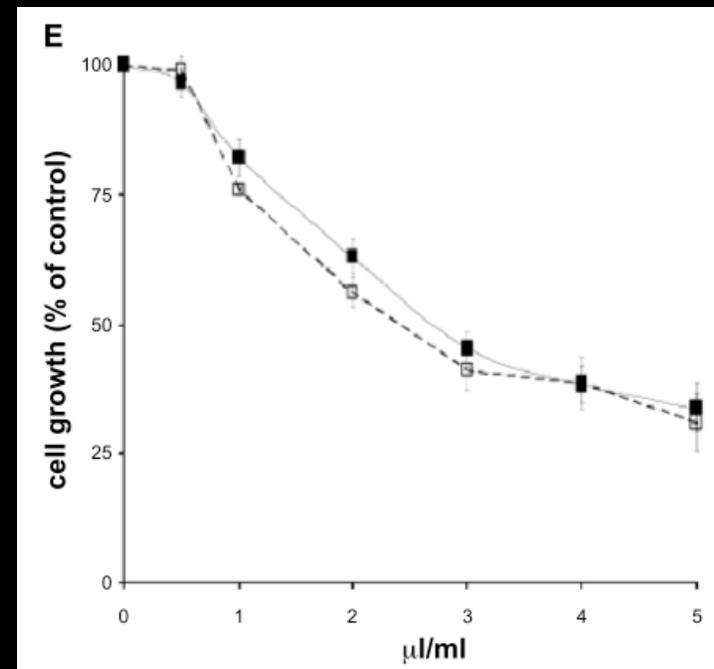
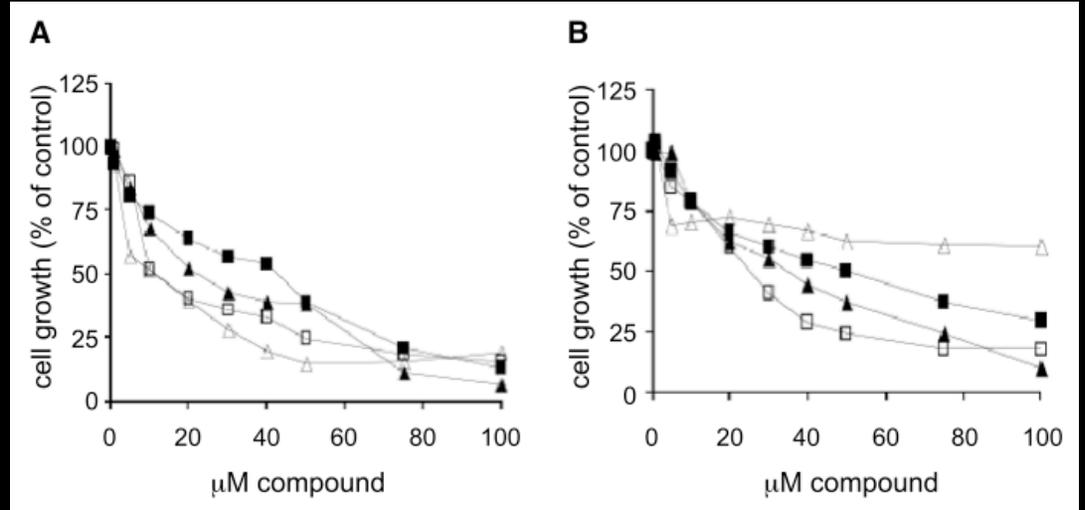
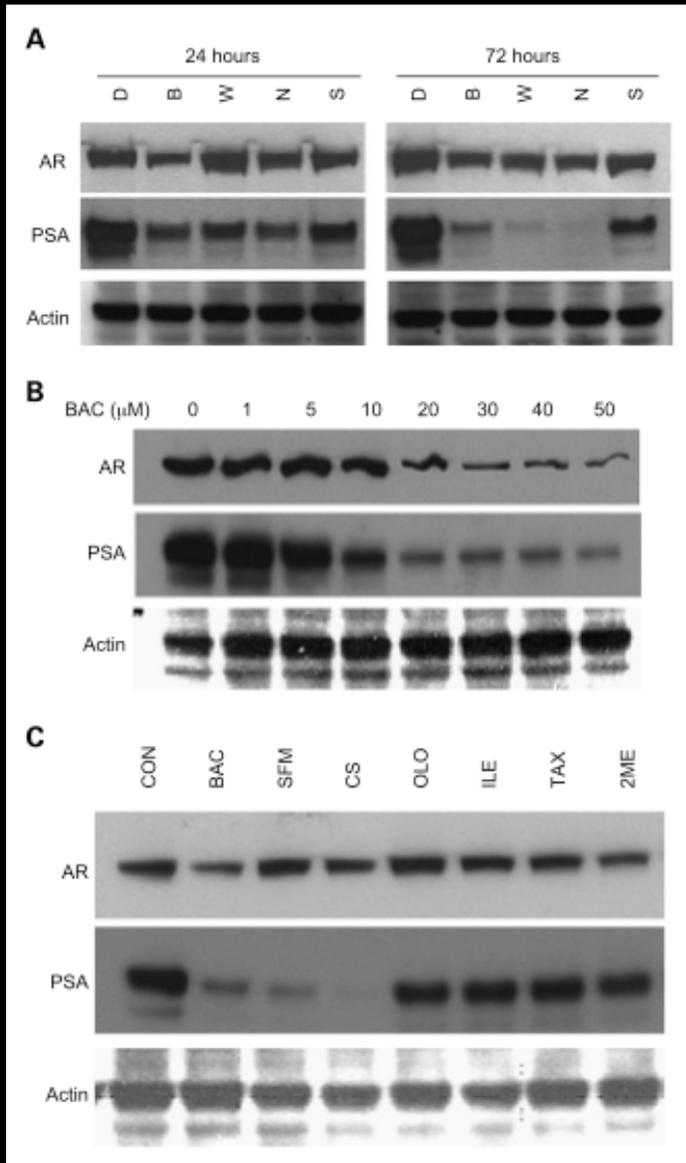
PSA



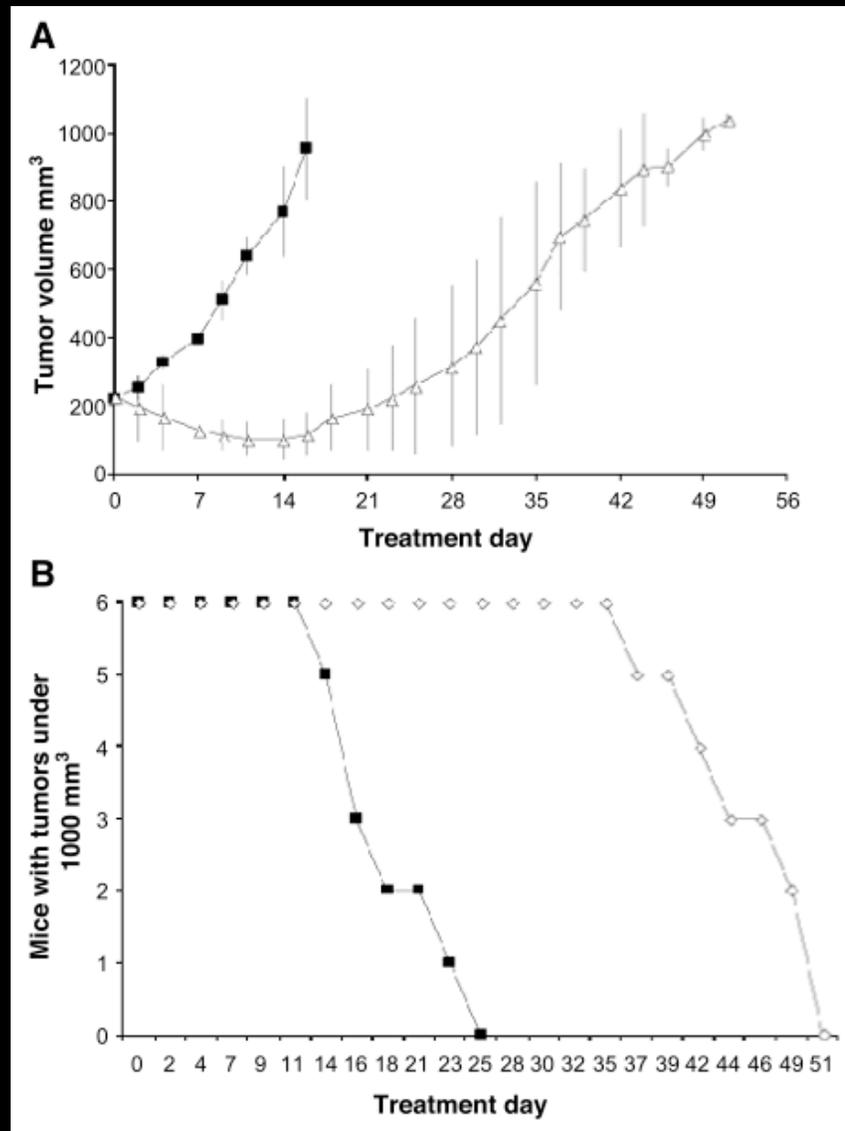
Scutellaria baicalensis: ID of Constituents



Scutellaria Baicalensis: Analysis of Constituents



Inhibition of Prostate Cancer Growth with Oral Baicalein



SUMMARY

- Some PC-SPES activity may be due to DES or other synthetic compounds.

SUMMARY

- Some PC-SPES activity may be due to DES or other synthetic compounds.
- Other molecular activities of PC-SPES are distinct from known Estrogens (DES).

SUMMARY

- Some PC-SPEs activity may be due to DES or other synthetic compounds.
- Other molecular activities of PC-SPEs are distinct from known Estrogens (DES).
- Mechanisms of activity may be due to down-regulation of AR and inhibition of microtubules.

SUMMARY

- Some PC-SPEs activity may be due to DES or other synthetic compounds.
- Other molecular activities of PC-SPEs are distinct from known Estrogens (DES).
- Mechanisms of activity may be due to down-regulation of AR and inhibition of microtubules.
- Consider potential for interactions with conventional therapies targeting AR or tubulins (e.g. Taxanes)

SUMMARY

- Some PC-SPEs activity may be due to DES or other synthetic compounds.
- Other molecular activities of PC-SPEs are distinct from known Estrogens (DES).
- Mechanisms of activity may be due to down-regulation of AR and inhibition of microtubules.
- Consider potential for interactions with conventional therapies targeting AR or tubulins (e.g. Taxanes)
- Active herbs include Scutellaria and Glycyrrhiza

SUMMARY

- Some PC-SPES activity may be due to DES or other synthetic compounds.
- Other molecular activities of PC-SPES are distinct from known Estrogens (DES).
- Mechanisms of activity may be due to down-regulation of AR and inhibition of microtubules.
- Consider potential for interactions with conventional therapies targeting AR or tubulins (e.g. Taxanes)
- Active herbs include Scutellaria and Glycyrrhiza
- Baicalin represents one specific chemical constituent with biological activity.

Thoughts....

- The evaluation of botanicals (and other CAM) should be performed in a rigorous, systematic manner.

Thoughts....

- The evaluation of botanicals (and other CAM) should be performed in a rigorous, systematic manner.
- Buyer beware: little control over variability and activity of most preparations (e.g. saw palmetto). *Contamination* is another issue.

Thoughts....

- The evaluation of botanicals (and other CAM) should be performed in a rigorous, systematic manner.
- Buyer beware: little control over variability and activity of most preparations (e.g. saw palmetto). *Contamination* is another issue.
- Botanicals have both benefits and side-effects, just as do prescribed medications.

There is no short-cut or magic bullet.

Thoughts....

- The evaluation of botanicals (and other CAM) should be performed in a rigorous, systematic manner.
- Buyer beware: little control over variability and activity of most preparations (e.g. saw palmetto). *Contamination* is another issue.
- Botanicals have both benefits and side-effects, just as do prescribed medications.
There is no short-cut or magic bullet.
- There may be **novel** and **potent** anti-cancer agents contained within botanical preparations.

Thoughts....

- The evaluation of botanicals (and other CAM) should be performed in a rigorous, systematic manner.
- Buyer beware: little control over variability and activity of most preparations (e.g. saw palmetto). *Contamination* is another issue.
- Botanicals have both benefits and side-effects, just as do prescribed medications.
There is no short-cut or magic bullet.
- There may be **novel** and **potent** anti-cancer agents contained within botanical preparations.
- There are no data to indicate that whole 'natural' forms or extracts of botanicals perform 'better' than individual constituents.

ACKNOWLEDGEMENTS

- Work performed by Michael Bonham (MSTP student)
- MS assistance provided by Carlos Gardner and William Howald, UWMC.
- Xenograft work in collaboration with David Agus and Anna Galkin (Cedars-Sinai)
- Bruce Montgomery, Seattle VAMC
- Julian Simons, FHCRC

Work supported by funding from CaPCURE/PCF and DOD