

The Effectiveness of Natural Products for Women's Health

8th Annual NHRI Scientific Symposium





Presented by:

"Using Botanicals, Hedgehogs, and Estrogens in the Prevention of Human Disease"

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NIH Goals for Botanical Centers

To coordinate, strengthen and foster new research and training efforts in the field of medicinal botanicals.

Mission of the MU Center for Botanical Interaction Studies

- Provide an interdisciplinary, collaborative research and training environment, to establish evidence for the mechanisms of action of botanicals from five plant species:
- 1. Sambucus nigra L. [subsp. canadensis (L.) Bolli Elderberry
- 2. Lessertia frutescens (L.) Goldblatt & J. C. Manning Sutherlandia/"Cancer Bush"
- *3. Picrorhiza kurrooa* Royle ex Benth
- 4. Glycine max Merr., F. Soy
- 5. Allium sativum L. Garlic
- Examine FIVE antioxidant signaling and related pathways involving: Reactive Oxygen Species (ROS), Nitric Oxide (NO), Inflammation, Estrogen and Hedgehog signaling.
- Support research projects studying botanical modulation of: prostate cancer, cerebral ischemia/stroke and immune system, as well as pilot projects.
- Support research cores for botanical sourcing and identification, chemical analysis, animal models (transgenic and knock-out) and molecular interactions/signaling systems studies.

Shared Missouri Botanical Center Research Theme

Helping people believe an herbal medicine/dietary supplement will work for their ailment by showing them how the herb/supplement works at the molecular level.
Without a molecular mechanism some people tend not to believe that an herb can work even when it does!



Prostate Cancer Project Research Goals:

- Define cellular responses in cultured tumor cells when exposed to pure compounds associated with botanicals.
- Identify botanicals that reduce prostate tumorigenesis in the transgenic TRAMP mouse model.
- Profile cellular responses in animal tissues after dietary consumption of botanicals.
- Explain the predominance of certain tumor phenotypes (WDC versus PDC) in different TRAMP genotypes (ER/Keap1WT versus ERaKO versus ERbKO versus Keap1KO (antioxidant pathway transgenic) versus tissuespecific- hedgehog transgenics treated with the same dietary supplement.



Genistein Is the Major Phytoestrogen in Soy



Genistein

17β-estradiol

ERα Kd=2.6 nM ERβ Kd=0.3nM ERα Kd=0.13 nM ERβ Kd=0.12 nM

Kuiper, G.G.J.M., et al., 1997.

Molecular Mechanisms of Action of Genistein

- Estrogenic/Anti-estrogenic (Wang , 1996 Shenouda 2004, Day/Slusarz/Jackson 2012)
- Tyrosine kinase inhibitor (Akiyama, 1987; Peterson & Barnes, 1993; Sakla 2007)
- Antioxidant (Ansell 2004)
- Alters the activity of enzymes involved in steroid metabolism: 5a-reductase (Evans, 1995) 17b-hydroxysteroid dehydrogenase (Makela, 1995), aromatase (Kao, 1998), other P450 pathways
- DNA Methylation (Day 2002 Zhuang)
- ERR (Wei Zhou 2006 Jinghua Liu 2009 Starkey, Lu)
- Hedgehog-signaling pathway (Sakla/Shenouda/Slusarz/Drenkhahn 2010, Jackson, Lu, Lin, Li)

Evidence for Cancer Protective Effects of the Soy Phytoestrogen Genistein

- Cancer Incidence Epidemiology
 - Breast Cancer: 4-5 times lower incidence in Asian cultures
 - **Prostate Cancer: USA** has 4-5 times the mortality rate of Japan
- Dietary intake
 - Asian Cultures:
 - 20-80 mg/day, plasma concentrations 50-800ng/ml (200nM-3µM)
 - Western Cultures:
 - 1-3 mg/day
- Experimental evidence
 - Cell culture models
 - Animal Cancer Models:

xenograft vs. carcinogen induced vs. transgenic

The TRAMP Mouse Model

TRansgenic Adenocarcinoma of Mouse Prostate

- The Transgene
 - PBTag
 - - 426 to +28 promoter region of probasin / SV40 T/t-antigen early region
 - Androgen regulated, prostate specific
- The Target
 - Abrogation of p53 and Rb gene function in the prostate
- Progression with metastasis to lymph nodes, lung, and bone



Greenberg et al. (Found on TRAMP webpage)

Prostate Tumor Progression in the TRAMP Mouse



Histology pictures kindly provided by Dr. Cynthia Besch-Williford

Overall Objectives of TRAMP Studies

> Determine the role of plant dietary supplements / phytoestrogens in the prevention of prostate cancer.

Determine the role of ERs in mediating the response to genistein and several other dietary supplements.



Dietary Genistein Reduces Tumorigenesis Via Estrogen Receptor-alpha (ERα) in the TRAMP Prostate Cancer model

Detailed Objectives

 Characterize prostate growth and tumor progression in double transgenic ERαKO/TRAMP mice fed a diet containing the soy phytoestrogen genistein

The Genistein – TRAMP 2x2 Animal Study Design

TRAMP mice	Casein diet	Genistein diet		
ERαWT	% with cancer	% with cancer		
ERαKO	% with cancer	% with cancer		

Purpose:

•Determine the role of the plant estrogen genistein in the prevention of prostate cancer (histology scores of 4-6) at 5 Months on casein- control or 300mg genistein / kg diets

•Determine the role of $ER\alpha$ in mediating the response to genistein.

The Genistein – TRAMP 2x2 Animal Study

% with Tumor	Casein diet	Genistein diet
ERαWT	72%	35%
Ε Rα KO	97%	96%

Treatment	Histology Stage (Pathological Score)					
(TRAMP)	(n)	HYP (2)	PIN (3)	WDC (4)	MDC (5)	PDC (6)
ERaWT-Casein	25	6 (24%)	1 (4%)	13 (52%)	0	5 (20%)
ERaWT-Genistein	29	12 (41%)	7 (24%)	6 (21%)	0	4 (14%)
ERa KO-Casein	29	1 (3%)	0	25 (86%)	1 (3%)	2 (7%)
ERa KO-Genistein	25	0	1 (4%)	23 (92%)	1 (4%)	0

∴ Genistein Prevents Prostate Cancer in ERaWT-TRAMP Mice but not in ERaKO-TRAMP mice

Genistein Conclusions

These differential results of genistein on prostate cancer incidence in WT and ER α KO TRAMP mice surprisingly suggest that genistein can exert its cancer protective effect through interaction with ER-alpha.

>TRAMP/ER α KO mice quickly get WDC but they have less PDC.

What about ER-beta's role in prostate cancer?

➤We have used ERβKO/TRAMP mice to analyze for additional effects of genistein and are using them to test for the ability of other phytoestrogens/dietary supplements to act through ER-beta.

The Role of ERs in Prostate Cancer

Mice were sacrificed at <u>5 months of age</u> and their tumors were removed and scored.

All mice were on the same casein (milk protein) diet.

ER beta+ / ER alpha- resulted in 1/4 the observed Poorly Differentiated Carcinoma.

ER alpha+ / ER beta- resulted in twice the prevalence of PDC.

	Diet	n	Tumor Stage					
Genotype			Non-Cancer		Cancer			
				НҮР	PIN	WDC	MDC	PDC
ERWT	Casein	175	2 (1%)	10 (6%)	41 (23%)	88 (50%)	0	34 (19%)
ERαKO	Casein	80	0	3 (4%)	4 (5%)	68 (85%)	1 (1%)	4 (5%)
ERβKO	Casein	51	0	0	13 (25%)	18 (35%)	0	20 (39%)

Slusarz, Jackson, Day, Lubahn et al. Endocrinology

Conclusions

ERαKO mice are protected against PDC, but have higher WDC incidence

ERβKO mice have higher PDC incidence, and have lower WDC incidence

Long term Lab Hypothesis

Inhibiting ER α and/or stimulating ER β will lower PDC incidence

Use of Estrogen Receptor selective ligands will mimic ER KO results and botanicals that select ER α vs. ER β will produce similar results.

Luteolin, genistein, phytosterols, oxysterols, statins. ERα specific antagonist **MPP**, ERβ specific agonist **DPN** What about the effects on PDC stage prostate cancer with the various botanical compounds that we have tested?

Very few botanical compounds decreased PDC

Effect of Phytoestrogens on Prostate Cancer

- EGCG, Spinacetin & Patuletin (from spinach extract) and high doses of Genistein, *reported* to prevent PDC. NIH RO1 on this (*we did not see these*)
- **Next with the Center grant:** 5 Center Botanicals in particular *Sutherlandia & elderberry & garlic*

How Do Phytoestrogens Prevent Prostate Cancer in TRAMP Mice?

Through which mechanism are these compounds working?

Through which mechanism are these compounds working?



Overall Hypotheses

Botanical compounds inhibit prostate cancer through the Hedgehog-signaling pathway AND

the estrogen receptor(s) are important in regulating this pathway



Sonic subgroup — Indian subgroup — Desert subgroup
Distances do not represent evolutionary divergence





Hedgehog Signaling

- Patched (Hh receptor) found in plants and bacteria
- Important for patterning and cell fate determination during embryonal development – mutations cause midline defects
- Aberrantly activated in skin, medullablastoma, bladder, pancreas, oesophageal, lung, colorectal, ALL, and **prostate** cancers
- Hedgehog pathway DNA mutations have been found in several of these cancers

Hedgehog Signaling

- What is cyclopamine
 - Teratogenic compound isolated from corn lily *Veratrum californicum*

• caused cyclopia in sheep



cyclopamine



Veratrum californicum (Hellebore):



• Inhibits the Hh signaling pathway

Hedgehog-Signaling Pathway



Cyclopamine treatment in mouse prostate cancer xenografts cures the cancer



PC3 xenografts

KARHADKAR et al. *Nature* **431**, 707 - 712 (07 October 2004)



PC3 xenografts

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KARHADKAR et al. Nature 431, 707 - 712 (07 October 2004)

Cyclopamine treatment in mouse xenografts cures the cancer



Rat prostate cancer cell lines:

AT6.3 – highly metastatic

AT2.1 – poorly metastatic

KARHADKAR et al. Nature 431, 707 - 712 (07 October 2004)

Hedgehog Inhibitors and Cancer Clinical Trials

- **Curis Genentech** Phase 2 clinical trials with colorectal cancer, *advanced basal cell carcinoma*, as well as a trial with various "treatment nonresponsive" advanced solid epithelial tumors (GDC-0449) **Approved as** *vismodegib* / trade name **Erivedge in the spring of 2012**
- Infinity AstraZeneca (IPI-926)
- Exelixis Bristol-Myers Squibb (XL139) (BMS-833923)
- Novartis (LDE225)
- **Pfizer -** (PF-04449913)

Hedgehog Signaling

•Before



•After 2 months of GDC-0449



•Before

•After 5 months of GDC-0449





Hedgehog Signaling

•Partial response and/or resistance to 1st generation Hh pathway inhibitors?

•Rudin, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. New England Journal of Medicine. 2009

•Van Hoff, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. New England Journal of Medicine. 2009

•Yauch, et al. Smoothened mutation confers resistance to a hedgehog pathway inhibitor in medulloblastoma. Science. 2009

Through which mechanism are these phytoestrogen compounds working?



Hypothesis

The Hedgehog-signaling pathway is activated in our models:

The TRAMP mouse prostate cancer model, as well as in <u>both</u> human prostate cancer and mouse TRAMP prostate cancer cell lines.

Is there crosstalk between Estrogen and Hedgehog-Signaling Pathways?

Reported Crosstalk between ER and Hedgehog Pathways

- Estrogen influences hedgehog signaling in the thymus -Li, 2002
- Estrogenization of neonatal rat prostates differentially altered expression of various hedgehog proteins in the pathway *Pu & Prins, 2004*
- E-cadherin is target of Hh pathway via Snail *Cano 2000; Fearon 2003*
- E-cadherin is regulated by ER via MTA3 and Snail *Fearon, 2003*
- Ihh is target of PR (which is ER regulated) in the mouse uterus Lee, 2006

Do Phytoestrogen treatments alter Hedgehog Pathway activity in TRAMP?

\$ and 2nd generation

Structures of Prostate Cancer Botanical Compounds





Summary

7 phytoestrogens at pharmacological concentrations are able to inhibit hedgehog signaling in prostate cancer cell lines

[Anna Slusarz, Sara Drenkhahn, et al. Cancer Research 2010]

New Botanicals Can Reduce Hedgehog Signaling in Stimulated Shh Light II Cells



Sutherlandia

Sutherlandia is an old remedy for cancer & is now also used as an immune booster for AIDS patients.

> FABACEAE (PEA & BEAN FAMILY) Lessertia frutescens SUTHERLANDIA (E) UMNWELE (X) KANKERBOS (A)

*



- *Sutherlandia* is used by traditional medical practitioners for their patients (usually in combination with other natural products)
- Lessertia frutescens ('Sutherlandia' / 'Unwele') is claimed to be an 'adaptogen', to modulate the immune system and to help individuals manage stress and symptoms of HIV infection. *Claims are made for treating cancers* and diabetes, etc.
- No modern clinical evidence for *Sutherlandia's* effectiveness in modulating stress, immune function or other claimed benefits in humans
- *Sutherlandia's* safety for HIV-positive adults is unknown and is a concern due to effects on CYP450 metabolism



A Randomized, Double-Blind, Placebo-Controlled Trial of *Lessertia frutescens* in Healthy Adults

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Participants: 25 adults who provided informed consent and had no known significant diseases or allergic conditions nor clinically abnormal laboratory blood profiles during screening.

Intervention: 12 participants randomized to a treatment arm consumed 400 mg capsules of *Sutherlandia* leaf powder twice daily (800 mg/d). 13 individuals randomized to the control arm consumed a placebo capsule. Each participant received 180 capsules for the trial duration of 3 mo.

Outcome Measures: The primary endpoint was frequency of adverse events; secondary endpoints were changes in physical, vital, blood, and biomarker indices.

Results: There were no significant differences in general adverse events or physical, vital, blood, and biomarker indices between the treatment and placebo groups (p > 0.05). However, participants consuming *Sutherlandia* reported improved appetite compared to those in the placebo group (p = 0.01). Although the treatment group exhibited a lower respiration rate (p < 0.04) and higher platelet count (p = 0.03), MCH (p = 0.01), MCHC (p = 0.02), total protein (p = 0.03), and albumin (p = 0.03), than the placebo group, these differences remained within the normal physiological range, and were not clinically relevant. The *Sutherlandia* biomarker canavanine was undetectable in participant plasma.

Sutherlandia fractions separated by HPLC using methanol as mobile phase





Summary

- The Hedgehog signaling pathway is activated (*and inhibited by cyclopamine*) in TRAMP mice and the TRAMP-C2 cancer cell line. And in human LNCaP and PC3 prostate cancer cell lines.
- Treatment with high concentrations of phytoestrogens: Genistein, EGCG, Curcumin and Resveratrol, and at low concentrations with the new botanicals under study, like *Sutherlandia*, are able to inhibit hedgehog signaling in mouse and human prostate cancer cell lines. *In vivo?*

Conclusions/Future Studies

➢ Functional ERa and ERb are needed for genistein to exert its protective effects on WDC prostate cancer in TRAMP mice.

➢Continue to explore the role of various botanicals in regulating hedgehog signaling, in particular their components, as well as in whole plant extracts like *Sutherlandia* and **elderberry** from which pure compounds can be isolated.

➢ Botanicals are MUCH cheaper than cyclopamine and the recently FDA approved hedgehog inhibitor.

Set up a human clinical trial with various dietary supplements/herbs to treat/prevent **prostate**, or other cancers– perhaps basal cell skin cancer or small cell lung cancer or ?

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