SOY ISOFLAVONES IN PROSTATE CANCER PREVENTION, TREATMENT AND SURVIVORSHIP RESEARCH

Ömer Küçük, MD
Professor of Hematology, Medical Oncology and Urology
Winship Cancer Institute of Emory University
Atlanta, Georgia, USA
okucuk@emory.edu

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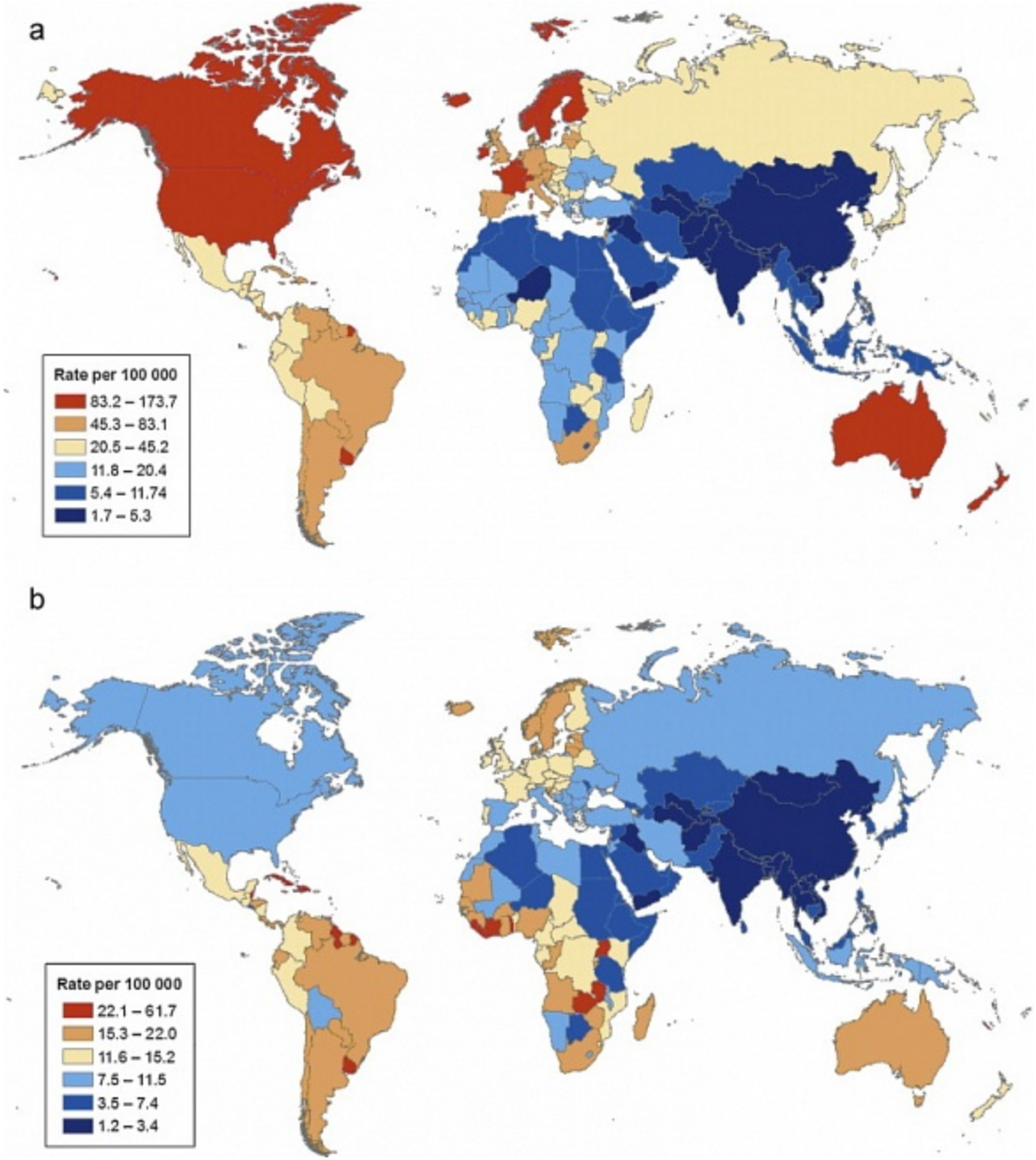
(a) Prostate cancer incidence (b) Prostate cancer mortality



Prostate cancer incidence and mortality worldwide

Prostate Cancer Incidence and Mortality Worldwide in 2008 – Summary

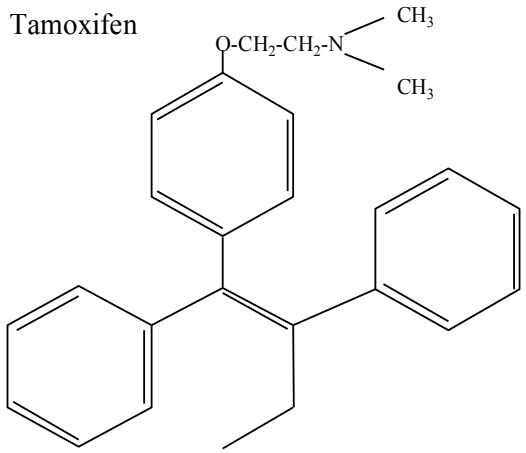
Estimated numbers (thousands)	Cases	Deaths
World	899	258
More developed regions	644	136
Less developed regions	255	121
WHO Africa region (AFRO)	34	24
WHO Americas region (PAHO)	334	76
WHO East Mediterranean region (EMRO)	12	9
WHO Europe region (EURO)	379	94
WHO South-East Asia region (SEARO)	28	19
WHO Western Pacific region (WPRO)	109	33
IARC membership (22 countries)	611	128
United States of America	186	28
China	33	14
India	14	10
European Union (EU-27)	323	71

Soy isoflavones and cancer

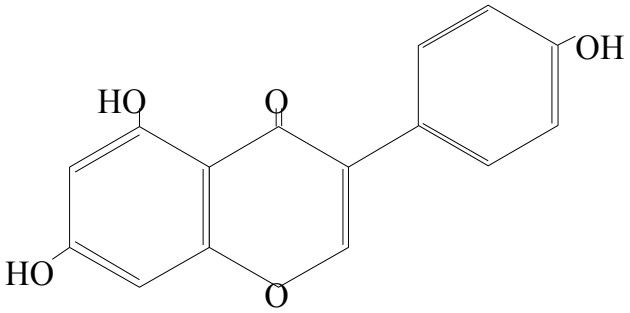
- Epidemiologic studies show an inverse association between dietary soy intake and cancer risk (breast, prostate, lung, and others)
- Genistein and daidzein are the most abundant isoflavones in soy
- Genistein has activity against a variety of cancer cells in culture, animal model and clinical studies

Soy Isoflavones

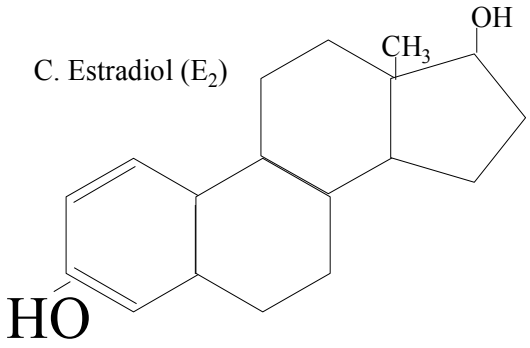
A. Tamoxifen



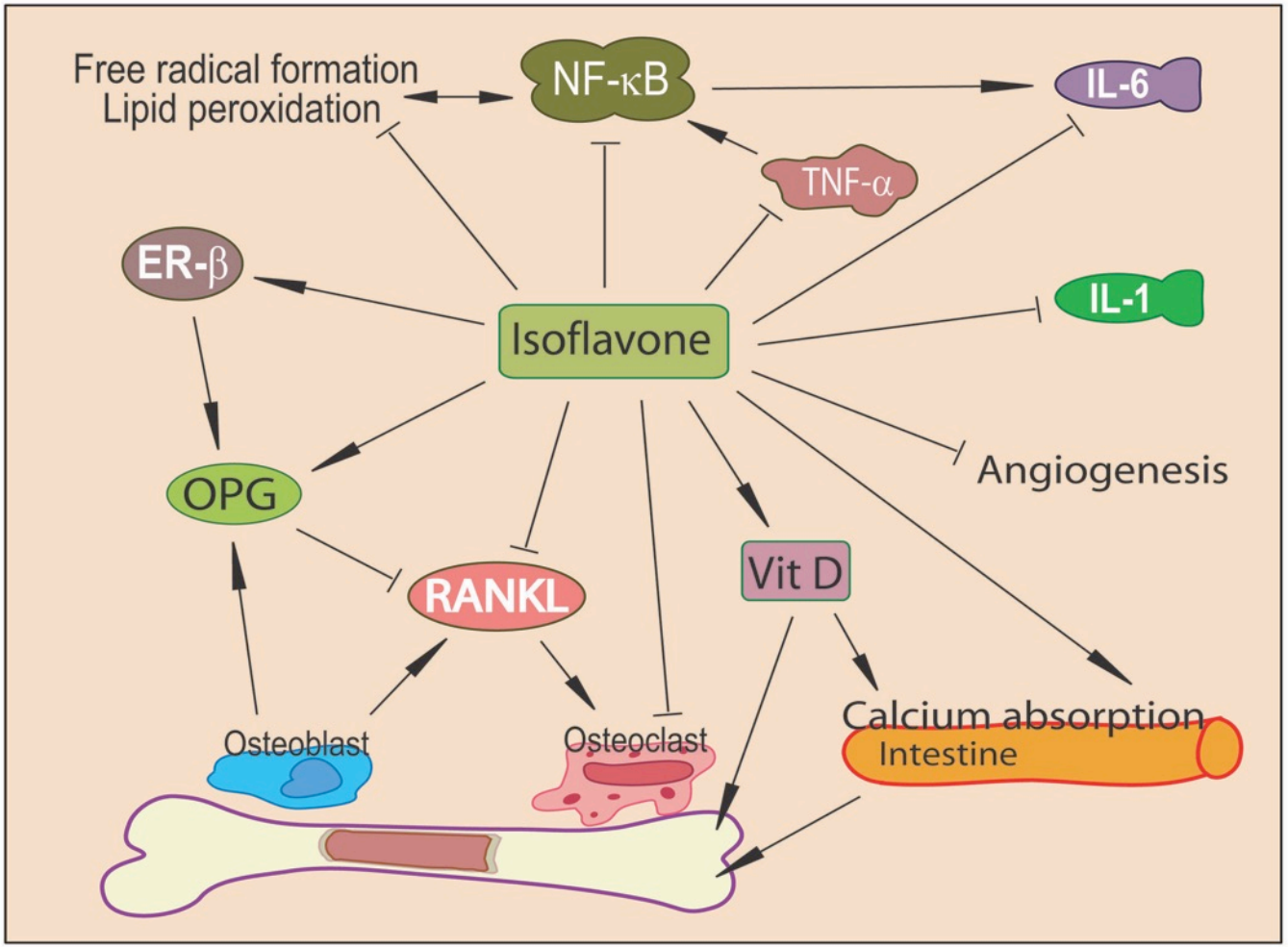
B. Genistein



C. Estradiol (E_2)



Genistein Has Pleiotropic Effects



Atmaca A, Kleerekoper M, Bayraktar M, Kucuk O. Menopause 15:748-57, 2008

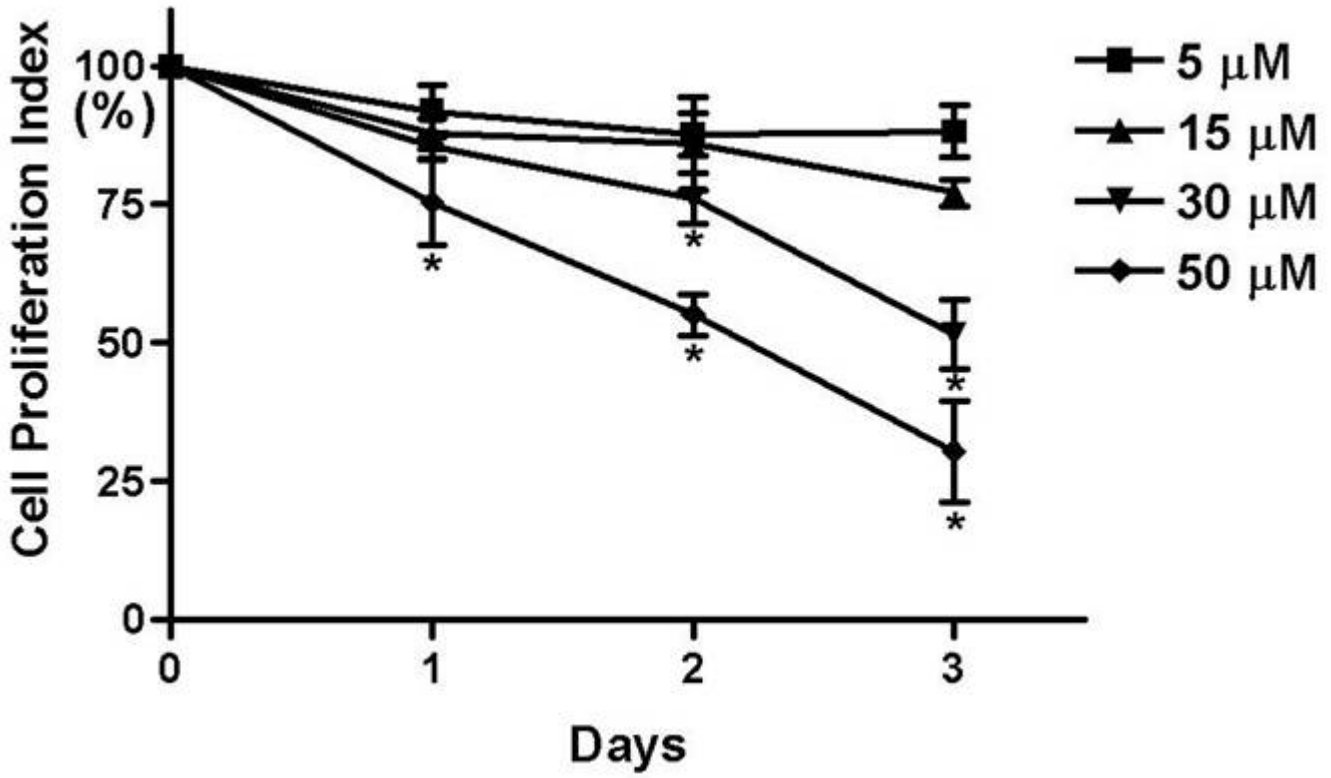
Genistein and Cancer

- Inhibits growth and induces apoptosis in Ca cells
- Growth inhibition mediated by G2/M cell cycle arrest and up-regulation of p21WAF1
- Down-regulates cyclin B1, CDKs, Bcl-2/Bcl-xL
- Up-regulates Bax expression and induces translocation of Bax to Mitochondria

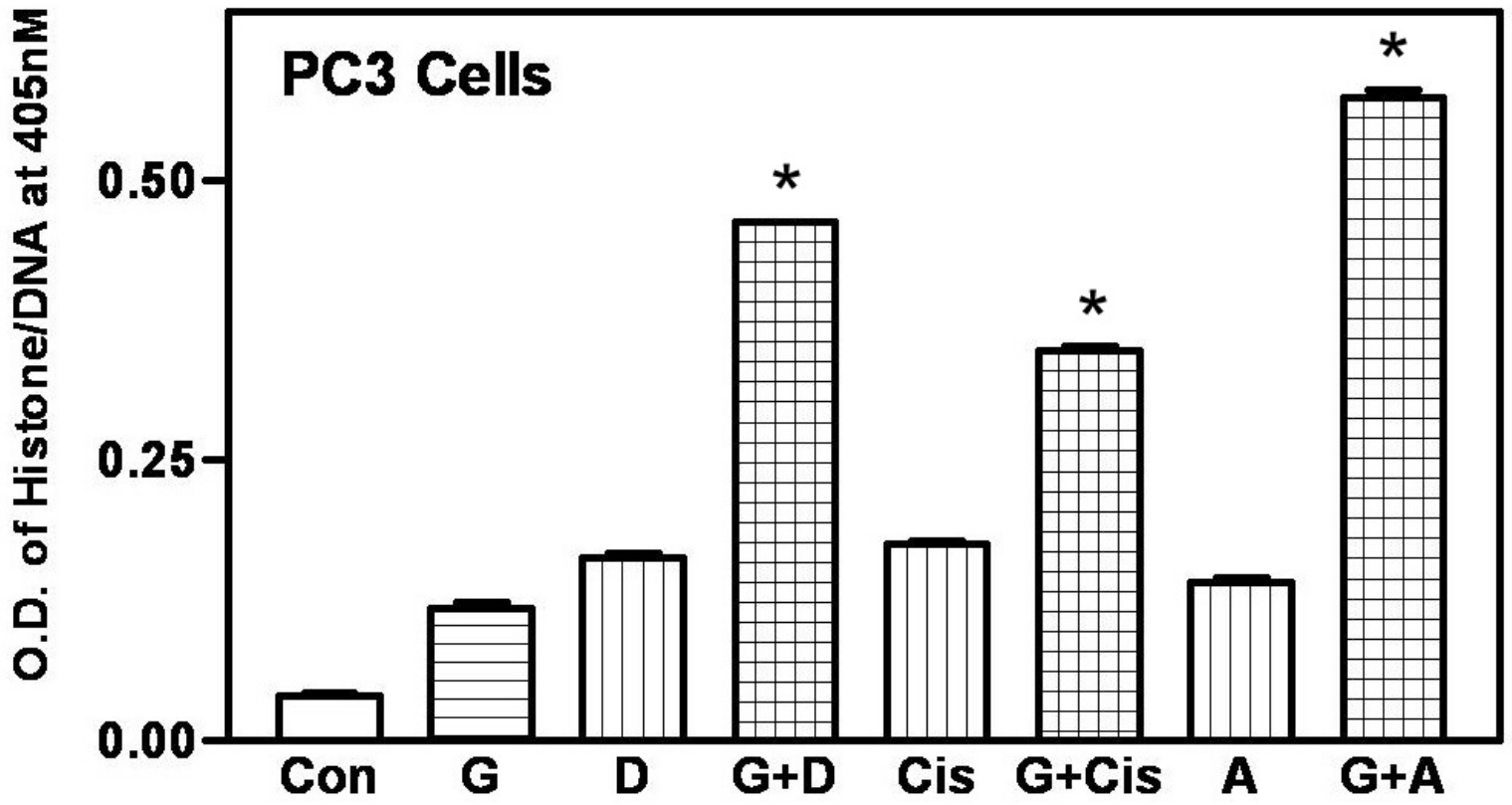
Genistein

- Down-regulates MMP-2, MMP-9, uPA, c-IAP and VEGF
- Inactivates Akt and NF-kB (by inhibiting IKK)
  - blocks nuclear translocation of p50 and p65
  - inhibits phosphorylation of IkBa
  - decreases MEKK1 kinase activity

Genistein and PC3 Proliferation

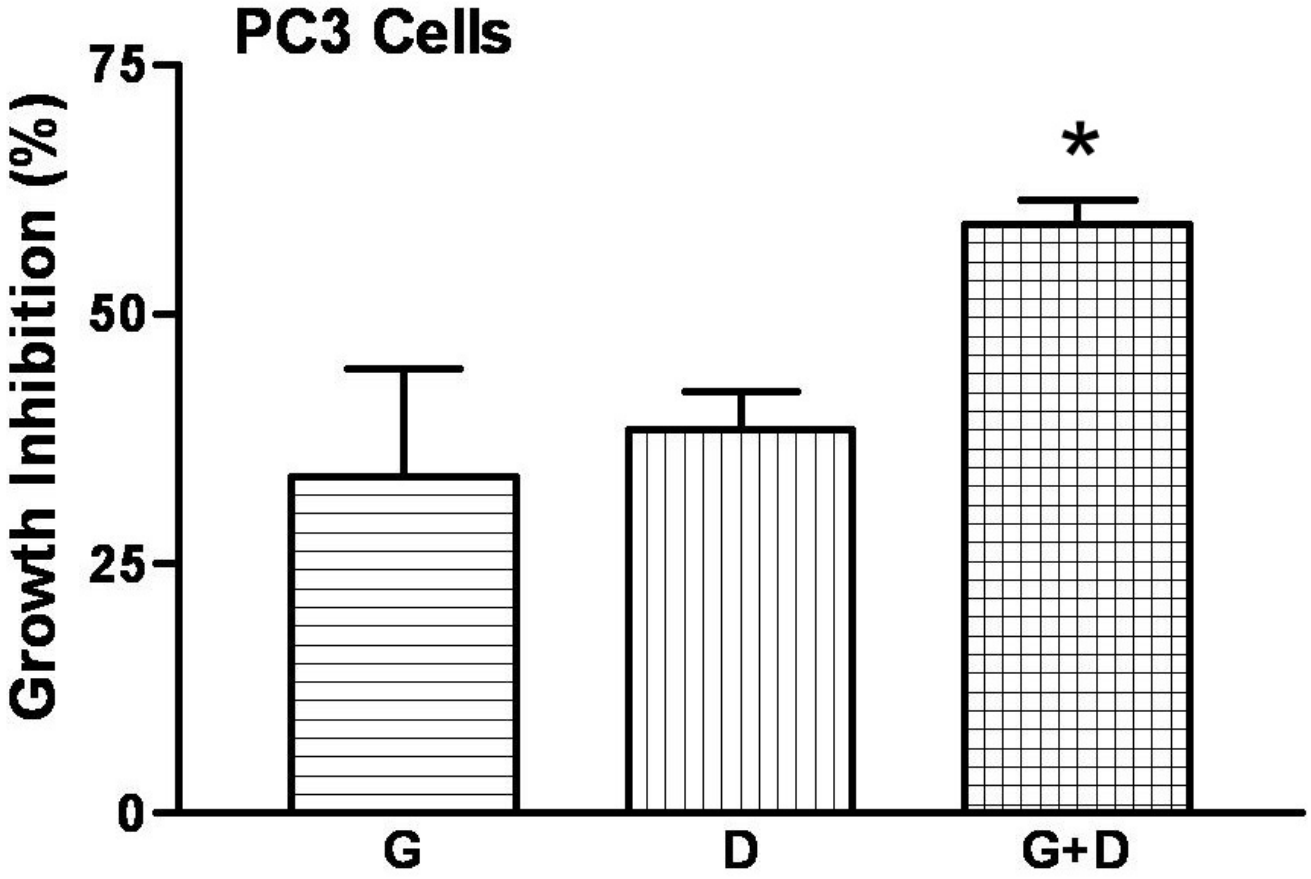


Apoptosis assay for PC3 cells treated with genistein, docetaxel, cisplatin, adriamycin, or combination



Con: Control; G: genistein; D: docetaxel; Cis: cisplatin; A: adriamycin
G+D: genistein followed by docetaxel; G+Cis: genistein followed by cisplatin.
G+A: genistein followed by adriamycin. *: $p < 0.01$

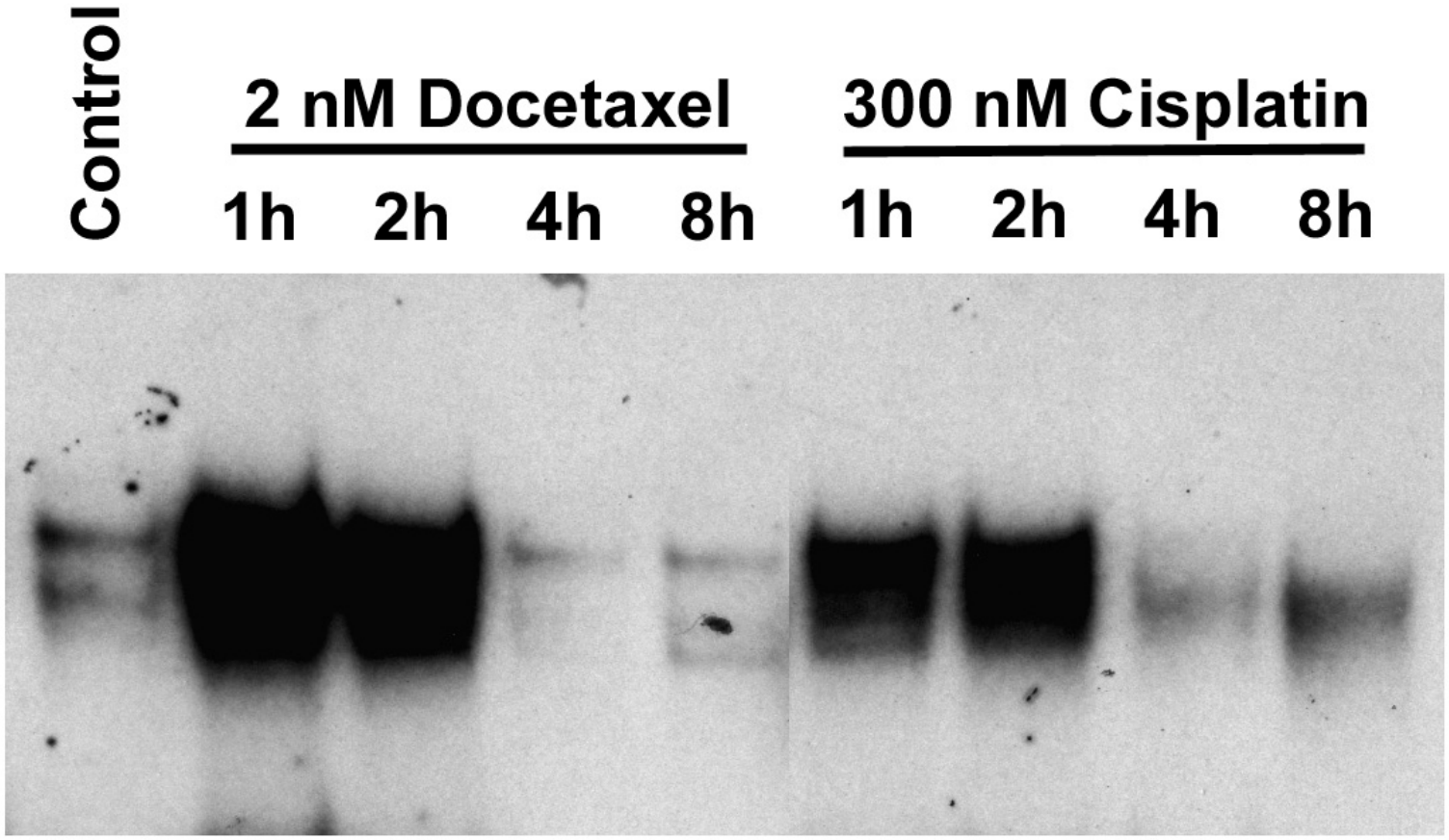
Growth inhibition in PC3 cells treated with genistein, docetaxel, or combination measured by MTT



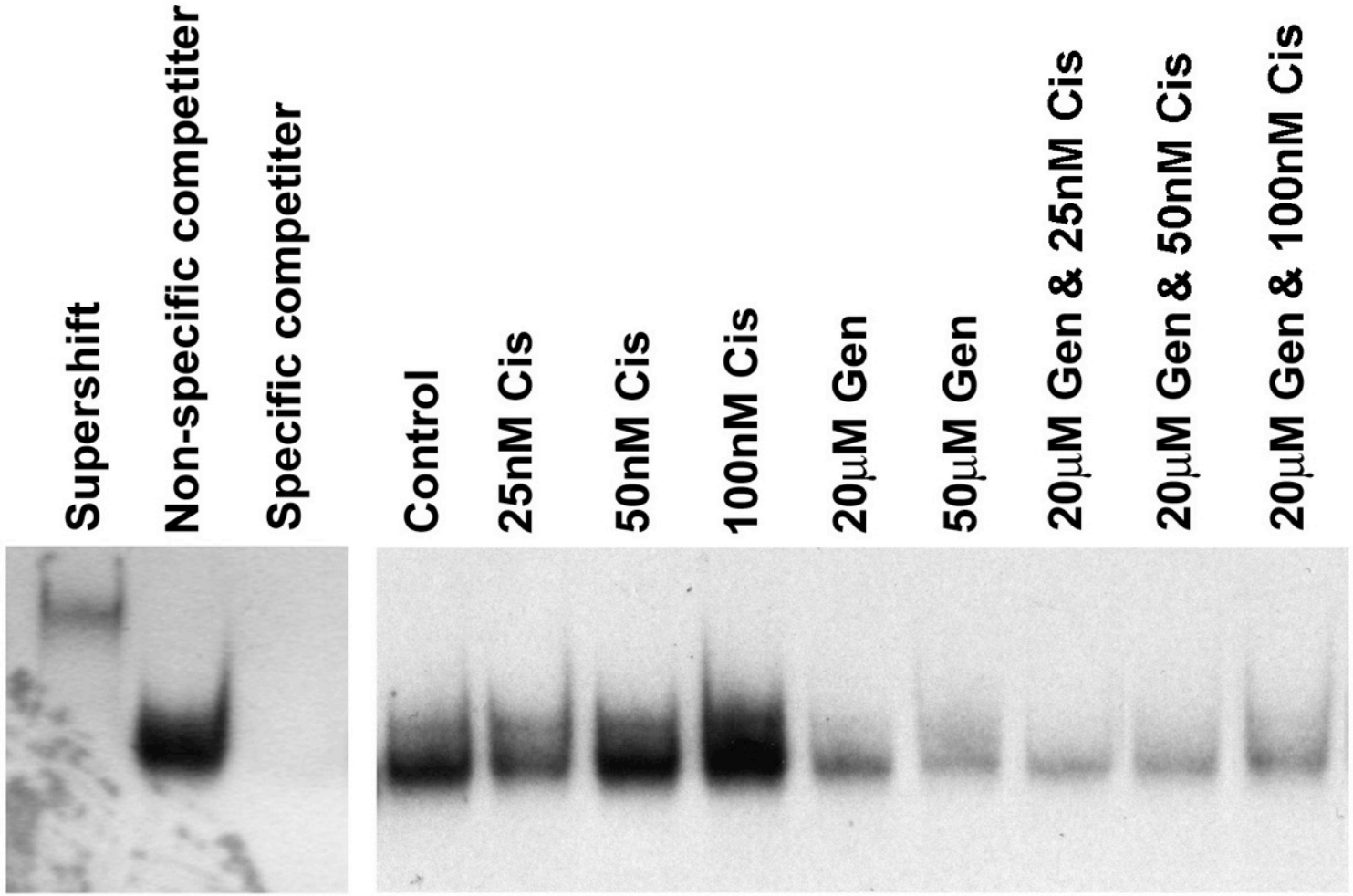
G: treated with 50 mM genistein for 48h; D: treated with 1nM docetaxel for 48h;
G+D: treated with 30 mM genistein for 24h followed by 0.5 nM docetaxel for 24h.

*: $p < 0.05$

EMSA for NF-kB activity in PC3 cells treated with docetaxel or cisplatin



EMSA for NF-kB activity in BxPC-3 cells treated with genistein, cisplatin, or combination



Cis: cisplatin; Gen: genistein.

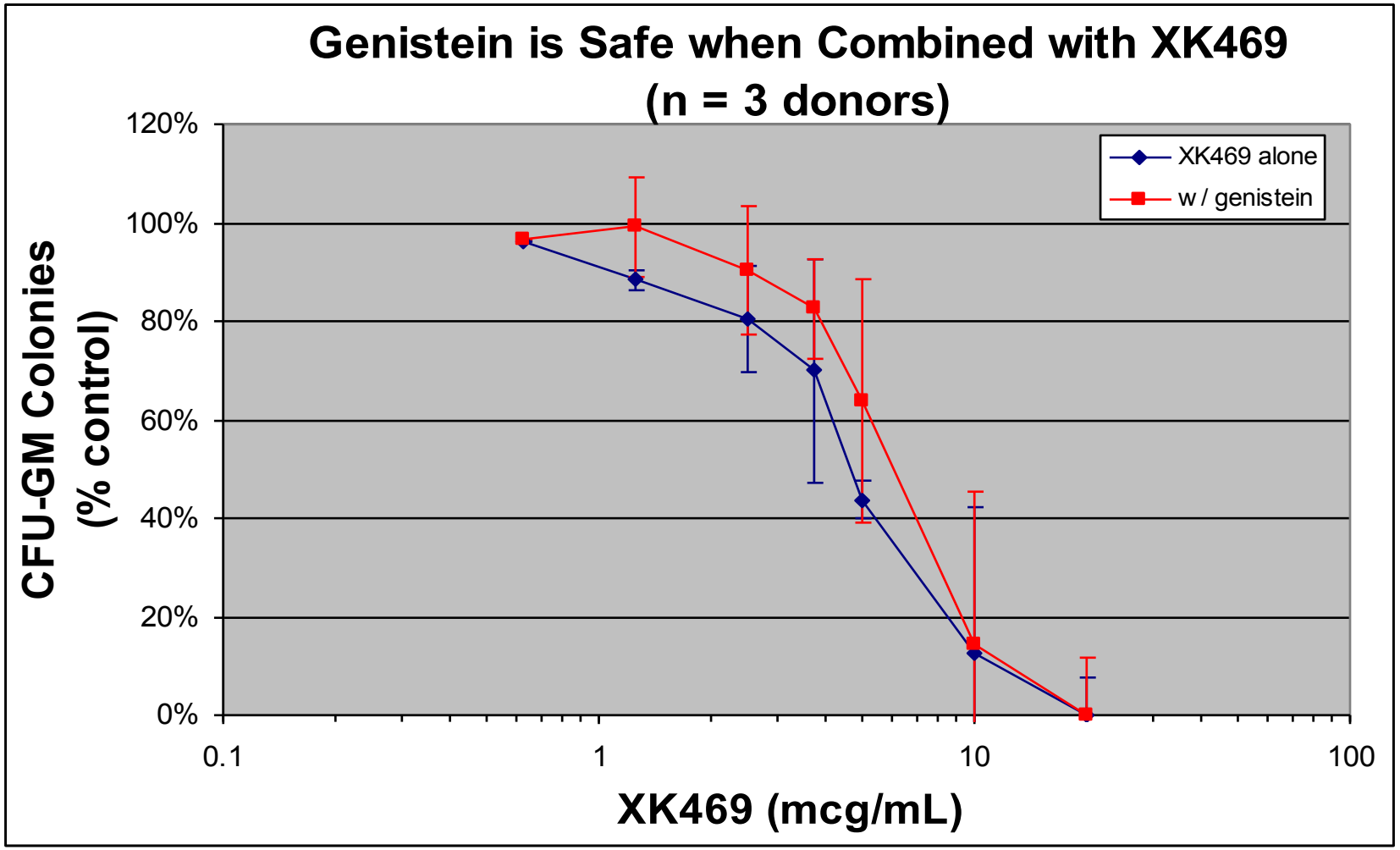
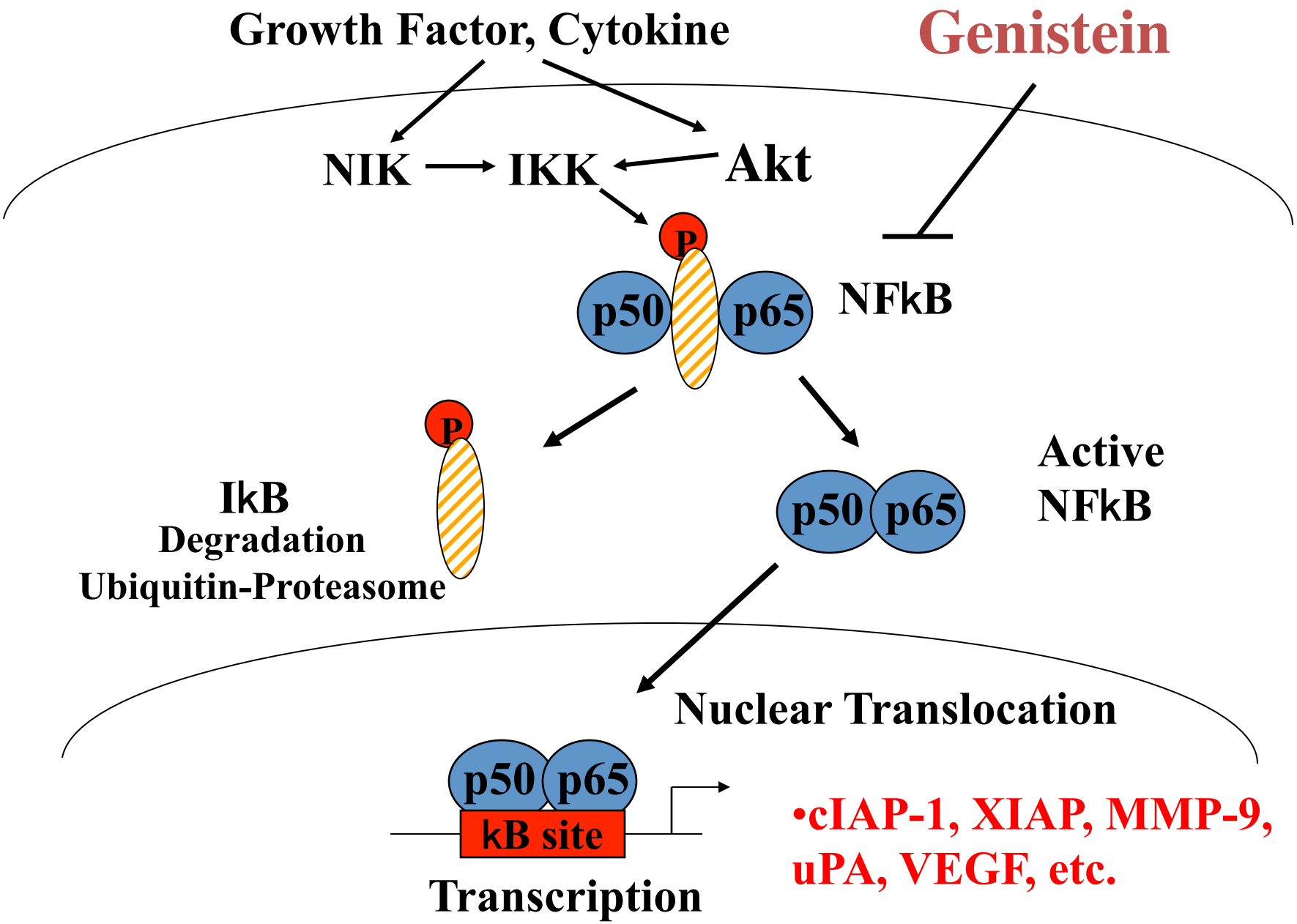
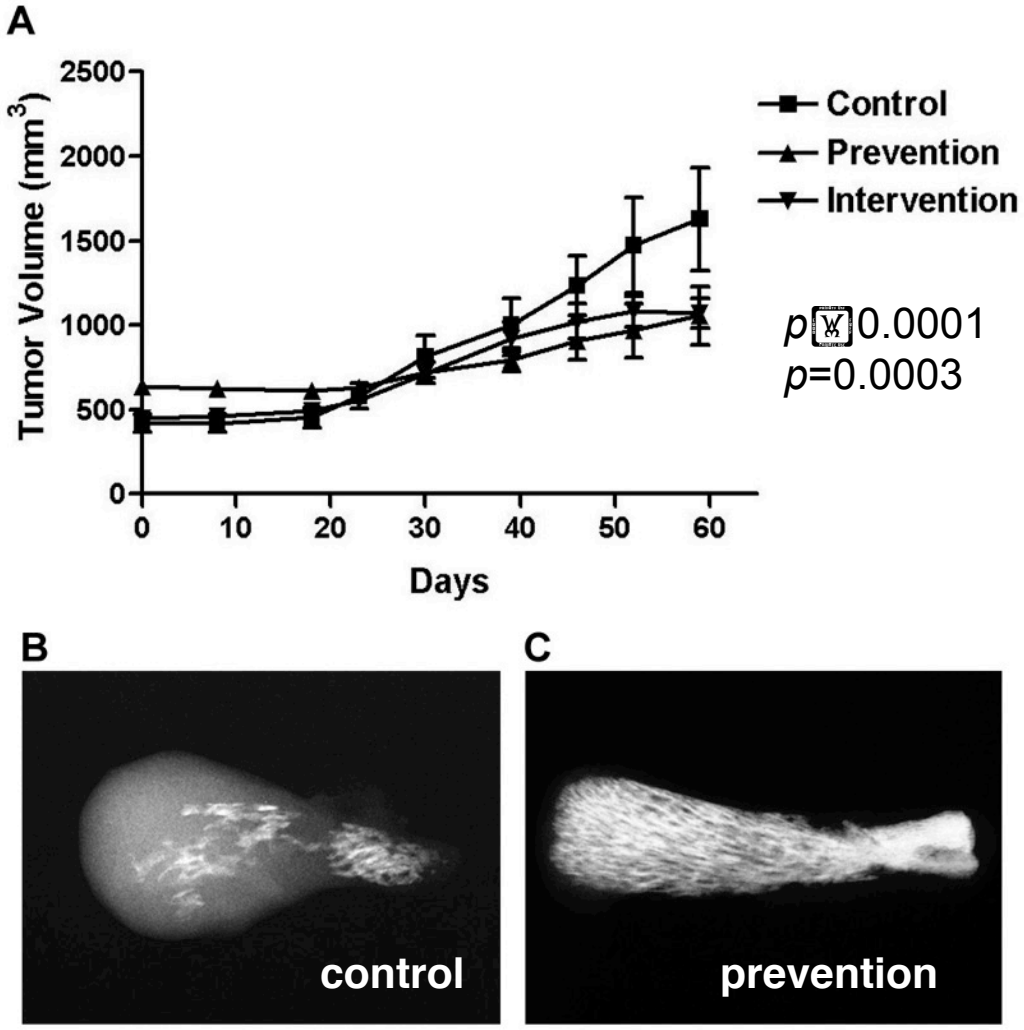


Figure 2. Three human bone marrows were processed to isolate the mononuclear cells, which were stimulated with rGM-CSF to produce clonogenic colonies of neutrophils and monocytes called CFU-GM. Toxicity of the investigational drug XK469 was quantified from inhibition of CFU-GM colony formation. The presence of 10-20 microM genistein did not change the potency of the toxic action of XK469 upon these hematopoietic cells.


Growth Factor, Cytokine
Genistein
NIK
IKK
Akt
P
p50
p65
NFkB
P
IkB
Degradation
Ubiquitin-Proteasome
p50
p65
Active
NFkB
Nuclear Translocation
p50
p65
kB site
Transcription
•cIAP-1, XIAP, MMP-9,
uPA, VEGF, etc.

Dietary Genistein and Experimental PC3 Bone Metastasis (Neoplasia 6:354-63, 2004)



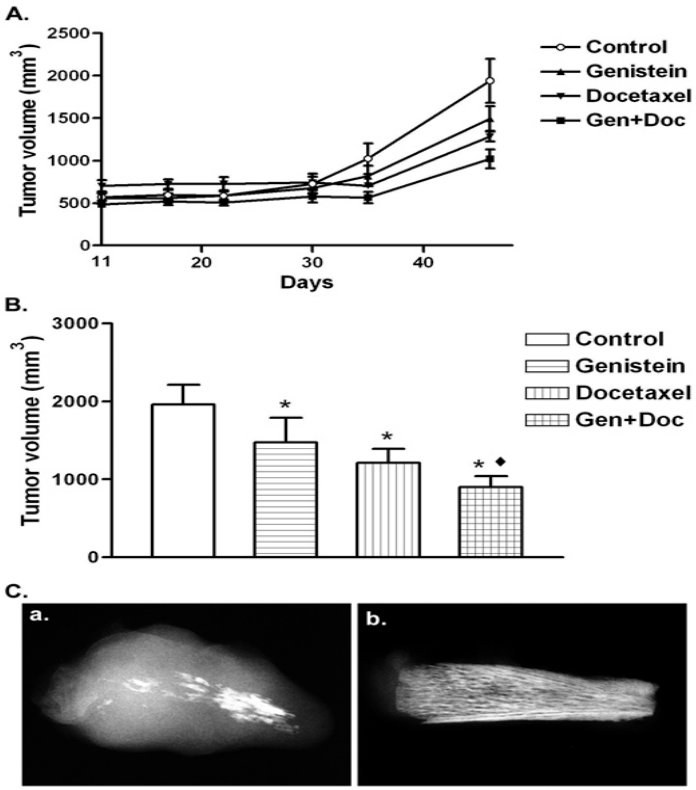


Fig-8: Genistein enhanced PC-3 bone tumor growth inhibition induced by docetaxel. A: Inhibitory effects of genistein and/or docetaxel on the growth of bone tumors formed by PC-3 cells in SCID-human mice. B: Comparison of the tumor volumes in each group on the day when all mice were sacrificed. (: p<0.01, Genistein vs Control, Docetaxel vs Control, Genistein+Docetaxel vs Control; ♦: p=0.01, Genistein+Docetaxel vs Docetaxel). C: Ex vivo bone tumor X-ray showed more osteolysis and tumor growth in control group (a) than in genistein treatment group (b).*

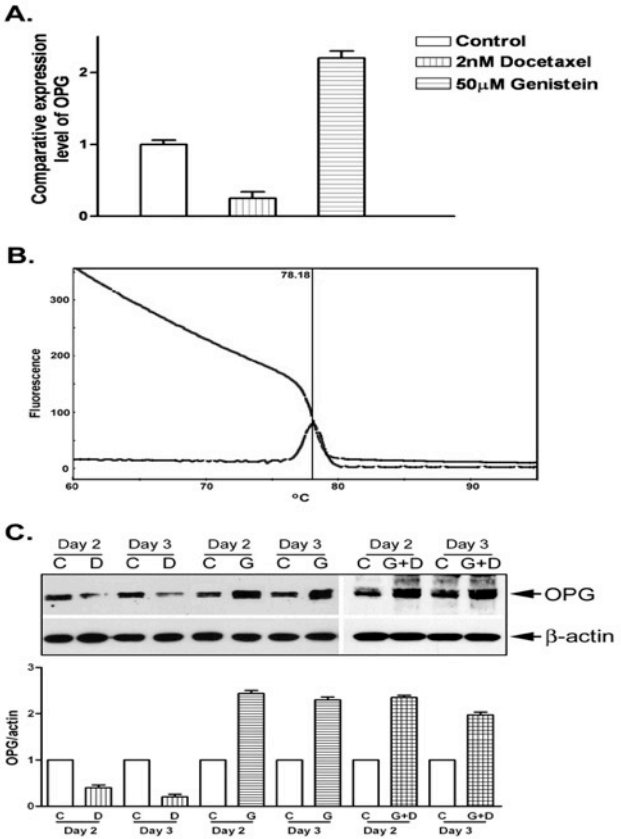
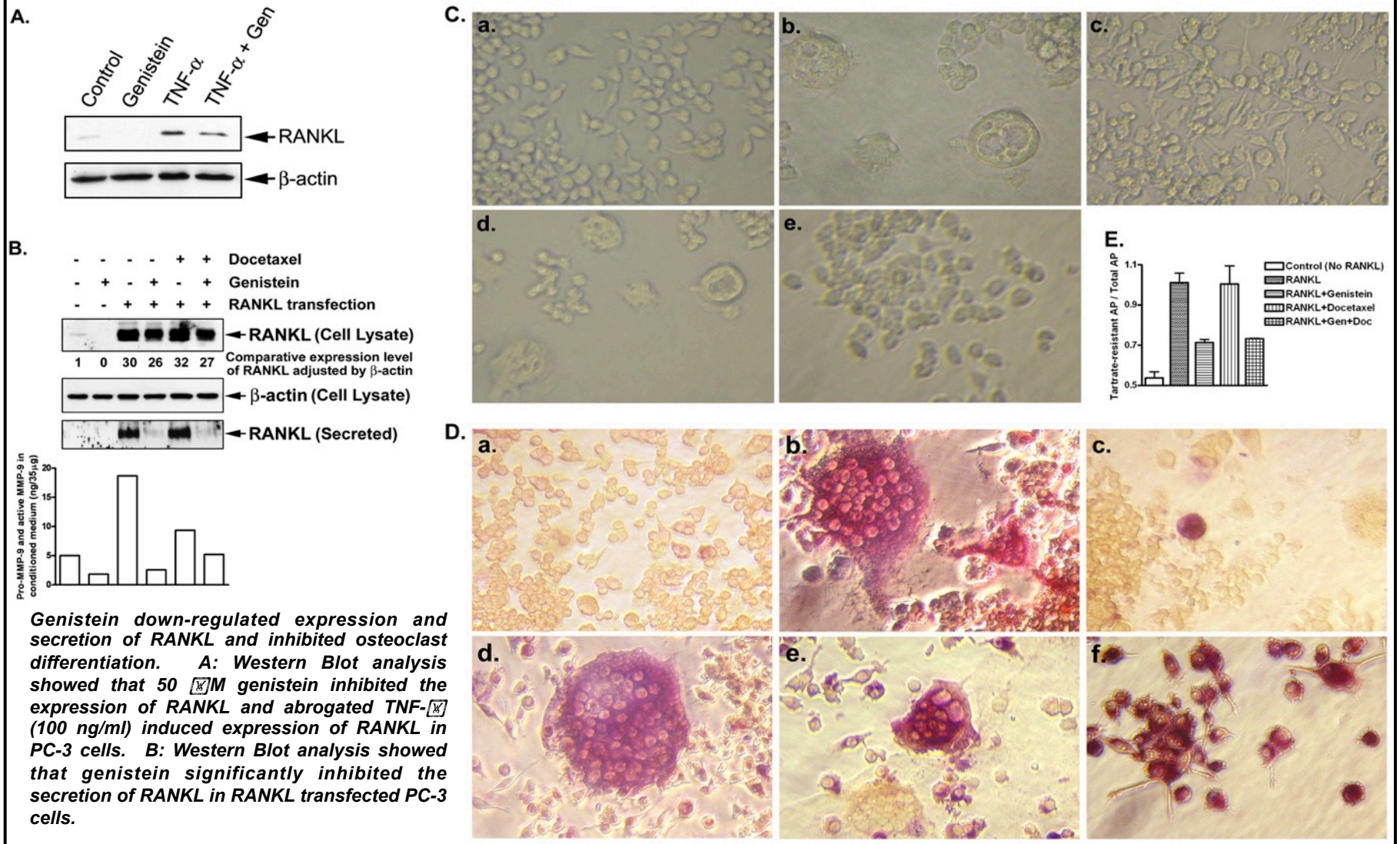


Fig-9: OPG expression was up-regulated by genistein and down-regulated by docetaxel. A: Real-time RT-PCR analysis of OPG mRNA expression in genistein or docetaxel treated PC-3 cells. B: Real-time RT-PCR melting curve showing the PCR product of OPG is pure (only one peak). C: Western Blot analysis of OPG protein expression in genistein and/or docetaxel treated PC-3 cells (C: Control; G: 50 μM Genistein treatment; D: 2 nM Docetaxel treatment; G+D: 30 μM Genistein and 1 nM docetaxel combination treatment).

Li Y et al. Cancer Res. 2006



Genistein down-regulated expression and secretion of RANKL and inhibited osteoclast differentiation. A: Western Blot analysis showed that 50 μM genistein inhibited the expression of RANKL and abrogated TNF-α (100 ng/ml) induced expression of RANKL in PC-3 cells. B: Western Blot analysis showed that genistein significantly inhibited the secretion of RANKL in RANKL transfected PC-3 cells.

MMP-9 activity assay showed that genistein significantly inhibited activity of MMP-9 secreted by RANKL-transfected PC-3 cells. C: Genistein inhibited RANKL-induced RAW264.7 cell differentiation to osteoclasts. The multinucleated osteoclasts were observed. (a. control, no RANKL added; b. treated with 100 ng/ml RANKL; c. treated with 100 ng/ml RANKL and 10 μM genistein; d. treated with 100 ng/ml RANKL and 0.5 nM docetaxel; e. treated with 100 ng/ml RANKL, 10 μM genistein, and 0.5 nM docetaxel; x200). D: Genistein inhibited RANKL-induced RAW264.7 cell differentiation to osteoclasts (TRAP staining; x200). In figures a to e, tartrate was added during staining. Multi-nuclei and purplish to dark red granules were observed only in osteoclasts. (a. control, no RANKL added; b. treated with 100 ng/ml RANKL; c. treated with 100 ng/ml RANKL and 10 μM genistein; d. treated with 100 ng/ml RANKL and 0.5 nM docetaxel; e: treated with 100 ng/ml RANKL, 10 μM genistein, and 0.5 nM docetaxel). In figure f, no tartrate was added during TRAP staining. The purplish granules indicated total acid phosphatase (tartrate-resistant and tartrate-sensitive acid phosphatase). RAW264.7 cells contain tartrate-sensitive acid phosphatase. E: Graph showed the ratio of tartrate-sensitive acid phosphatase versus total acid phosphatase. The value indicated the comparative amount of osteoclasts in each sample.

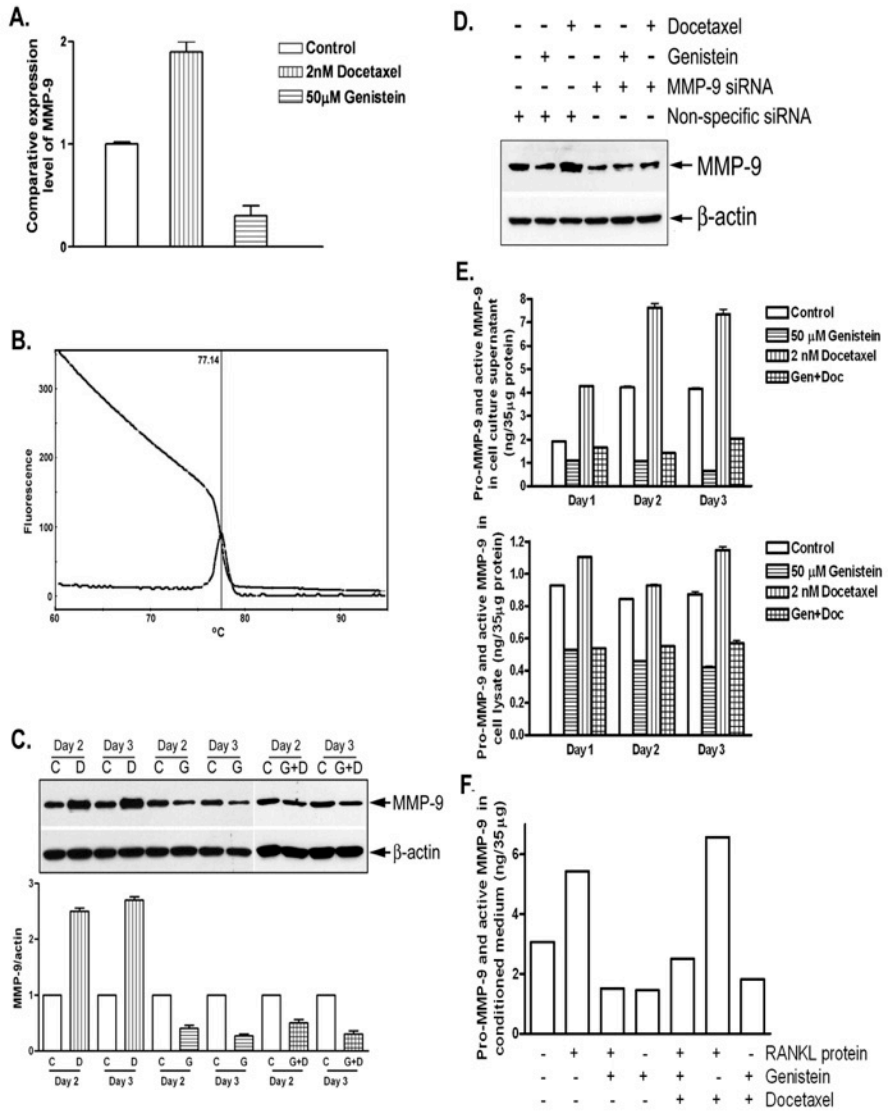


Fig-14: MMP-9 expression was up-regulated by docetaxel and down-regulated by genistein. A: Real-time RT-PCR analysis of MMP-9 mRNA expression in genistein or docetaxel treated PC-3 cells. B: Real-time RT-PCR melting curve showing the PCR product of MMP-9 is pure (only one peak). C: Western Blot analysis of MMP-9 protein expression in genistein and/or docetaxel treated PC-3 cells (C: Control; G: 50 μM Genistein treatment; D: 2 nM Docetaxel treatment; G+D: 30 μM Genistein and 1 nM docetaxel combination treatment). D: Western Blot analysis of MMP-9 protein expression in genistein or docetaxel treated PC-3 cells with or without MMP-9 siRNA transfection. E: MMP-9 activity assay showed that MMP-9 was up-regulated by docetaxel and down-regulated by genistein in PC-3 cell lysate and conditioned medium. F: MMP-9 activity assay showed that MMP-9 was up-regulated by docetaxel and down-regulated by genistein in conditioned medium of PC-3 cell and RANKL induced RAW264.7 cell co-culture.

Li Y et al. Cancer Res. 2006

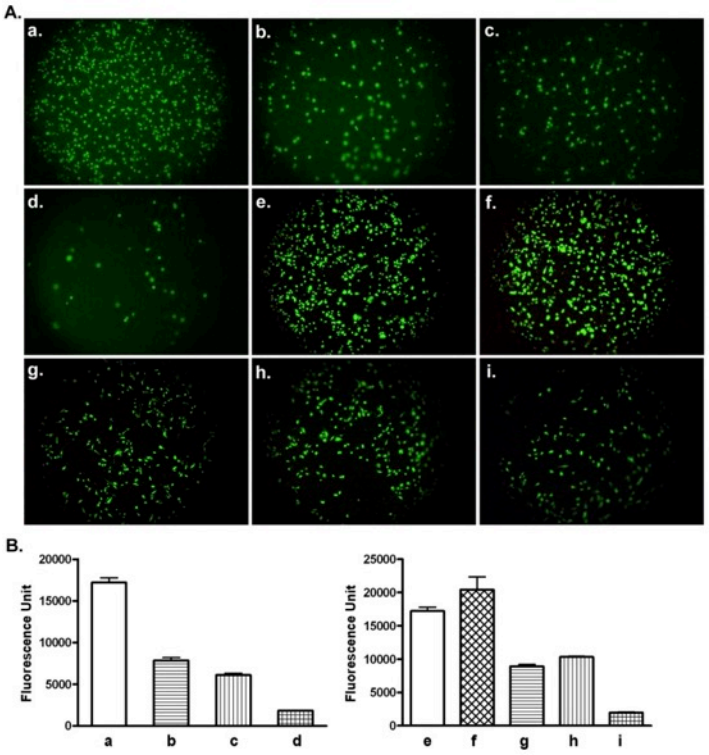
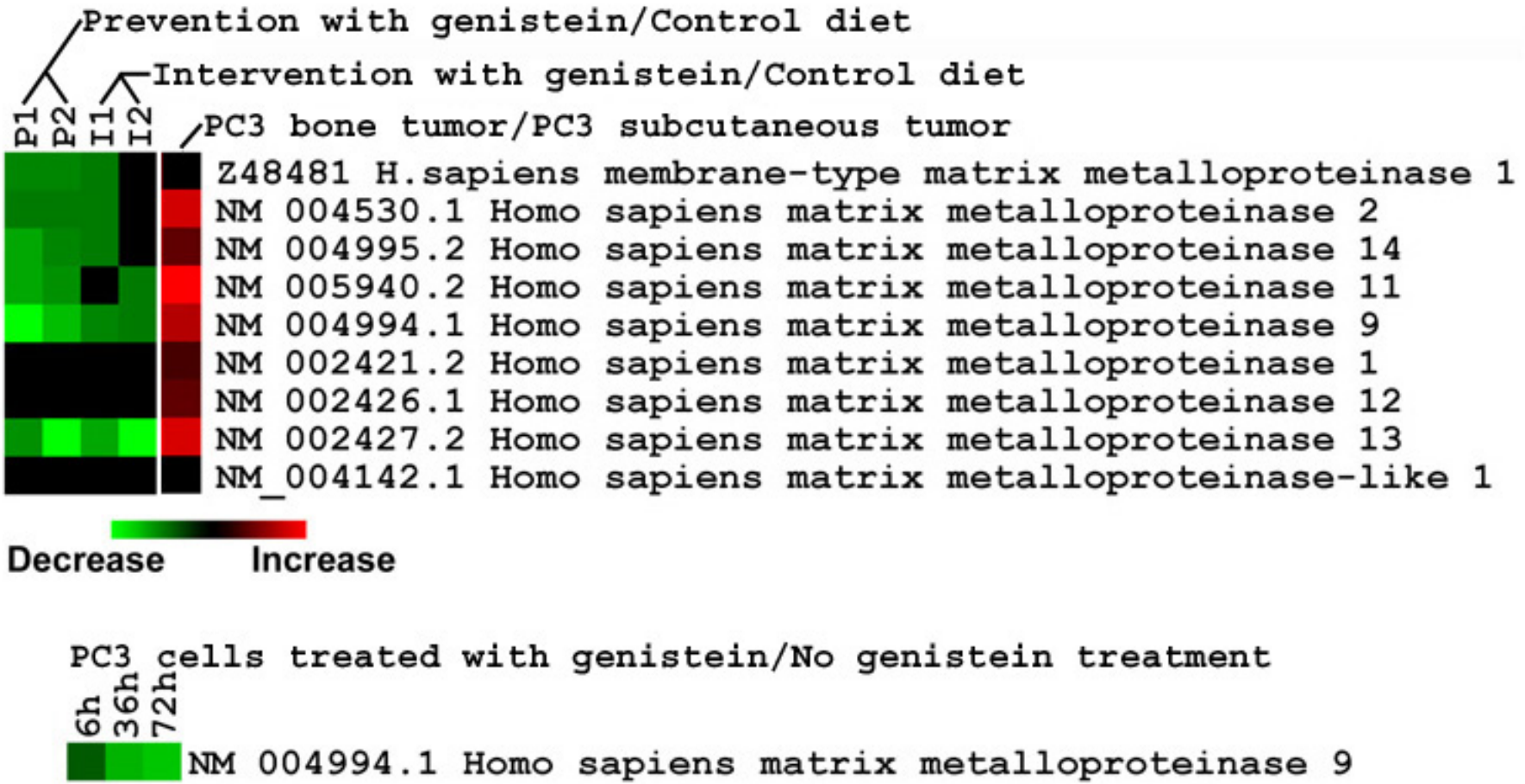


Fig-11: Genistein potentiated docetaxel-induced inhibition of PC-3 cell invasion. A: Invasion assay showed that combination treatment of genistein and docetaxel significantly inhibited the invasion of PC-3 cells through matrigel matrix membrane in PC-3 growth (a to d) or differentiated osteoclast growth (e to i) environment. (a. control; b. treated with 50 μM genistein; c. treated with 2 nM docetaxel; d. treated with 50 μM genistein and 1 nM docetaxel; e. control, no RANKL added; f. treated with 100 ng/ml RANKL; g. treated with 100 ng/ml RANKL and 50 μM genistein; h. treated with 100 ng/ml RANKL and 2 nM docetaxel; i: treated with 100 ng/ml RANKL, 50 μM genistein, and 1 nM docetaxel; x200). B: The graphs showed the value of fluorescence from the invaded PC-3 cells. The value indicated the comparative amount of invaded PC-3 cells.

Li Y et al. Cancer Res. 2006

Effect of Dietary Genistein on MMP Gene Expression in Experimental Metastasis



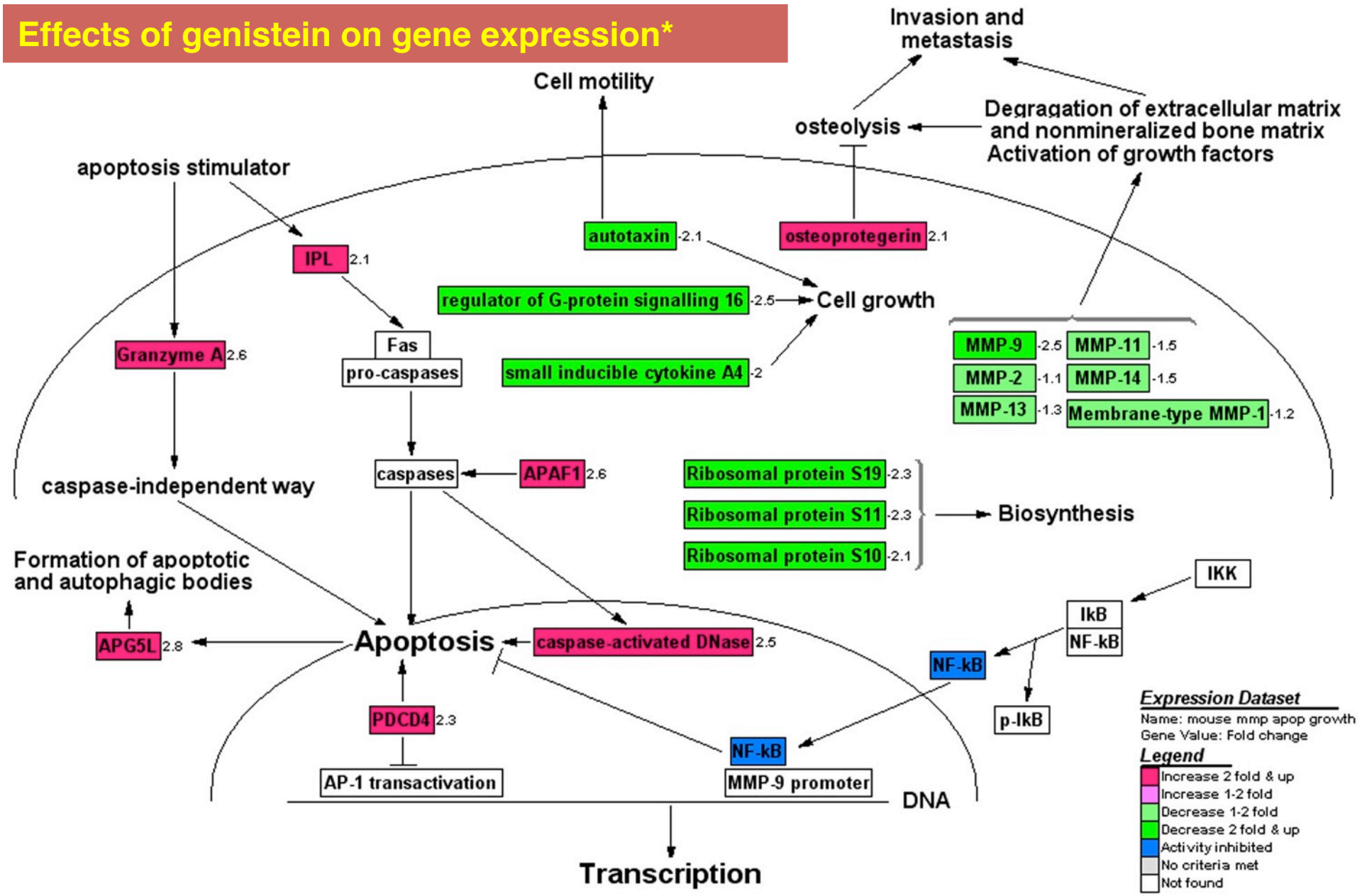
Affymetrix Human Genome U95 or U133A Array

Cluster Analysis According to Biological Function

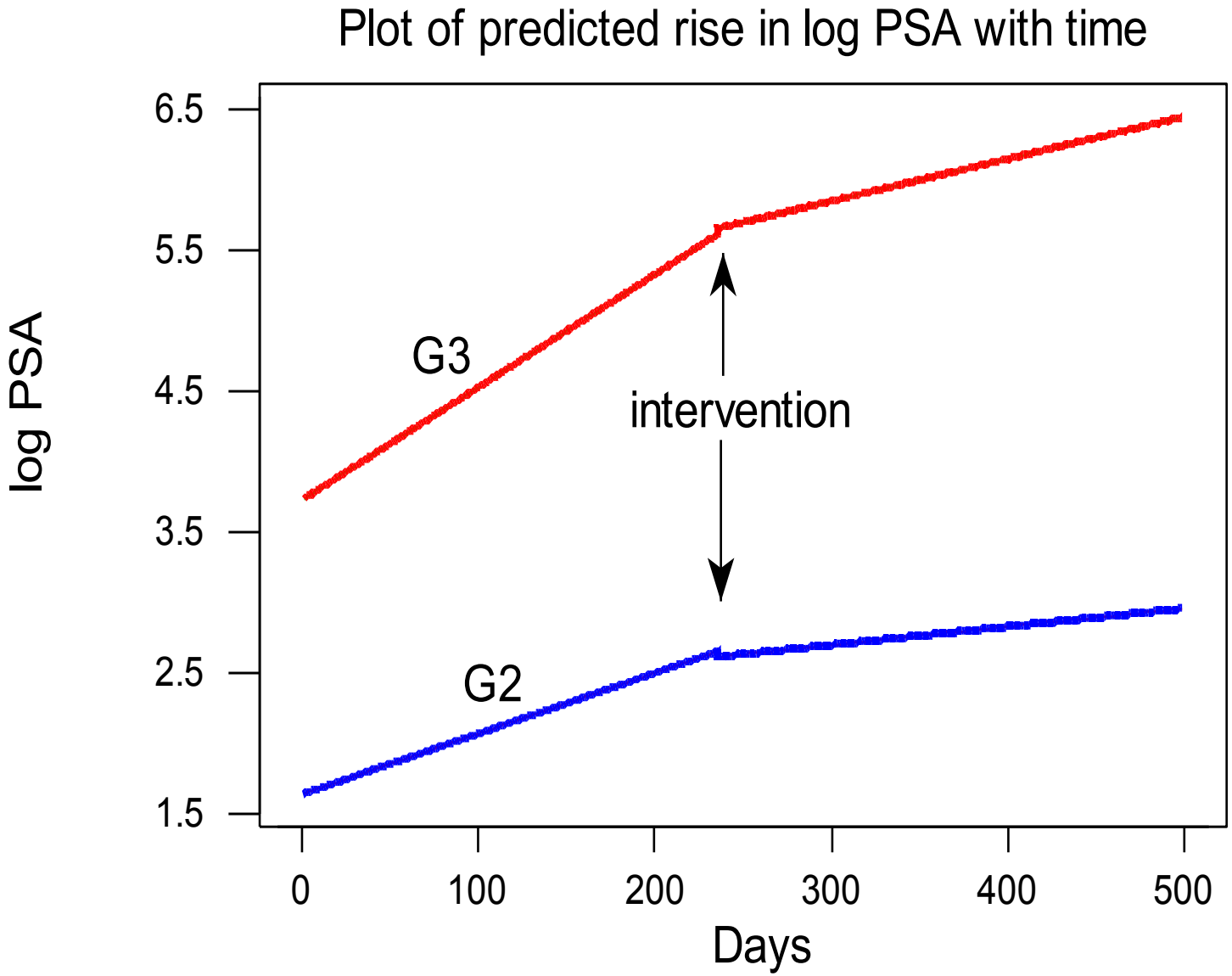
Numbers of altered genes in different categories in PC3 bone tumors after

Category	*Up*	*Down*
apoptosis	12	1
cell cycle arrest, negative regulation of cell proliferation and transcription	13	0
signal transduction, chemotaxis	10	7
regulation of transcription and protein biosynthesis	11	10
oncogenesis	8	4

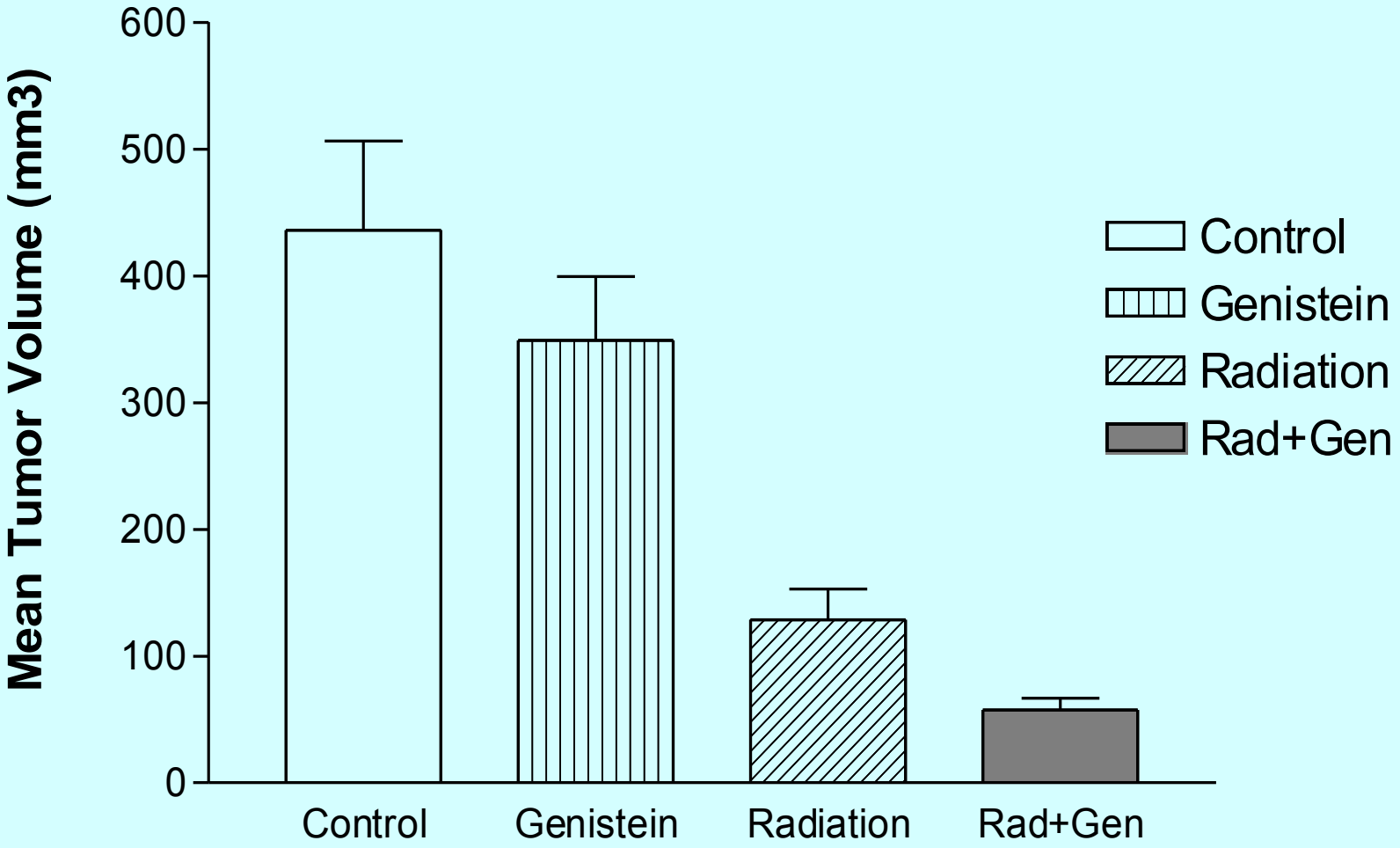
Effects of genistein on gene expression*



*Based on in vitro and in vivo gene profiling with and without genistein


Plot of predicted rise in log PSA with time
log PSA
6.5
5.5
4.5
3.5
2.5
1.5
G3
intervention
G2
0
100
200
300
400
500
Days

Treatment of PC-3 Prostate Tumors with Radiation + Genistein in Nude Mice



Genistein-Radiation Pilot Study

- 42 patients with prostate cancer
- Randomized, placebo-controlled, phase 2 study
- 20 patients received soy isoflavones 200 mg/day for 3 months, starting with the first day of radiation, and 22 received placebo
- QOL questionnaires given at 3 and 6 months

Study Patients

Group 1 (Soy)		**Group 2 (Placebo)**	
Median Age = 60 y		Median Age = 65 y	
8 T1c, 3 T2a, 2 T2b		10 T1c, 2 T2a, 1 T2b	
Median Pre PSA	3.7	Median Pre PSA	4.9
Median 4-6 month PSA	0.9	Median 4-6 month PSA	2
PSA decrease	75.7%	PSA decrease	59.2%

Genitourinary (GU) Toxicity

Soy	**3M** n=13	**6M** n=13	**Placebo**	**3M** n=13	**6M** n=14
GU toxicity			**GU toxicity**		
Leakage/Dripping of Urine	15.4% (2)	7.7% (1)	Leakage/Dripping of Urine	23.1% (3)	28.6% (4)
Big/Medium Problem with Frequency	38.5% (5)	0%	Big/Medium Problem with Frequency	38.5% (5)	7.1% (1)
Big/Medium Problem with Urgency	30.8% (4)	0%	Big/Medium Problem with Urgency	0%	0%
Function same as before RT or Better	92.3% (12)	92.3% (12)	Function same as before RT or Better	92.3% (12)	85.7% (12)

Erectile Function

Soy	**3 M** n=13	**6 M** n=13	**Placebo**	**3 M** n=13	**6 M** n=14
Erectile Function			**Erectile Function**		
Ability to Have Full Erections	69.2% (9)	77% (10)	Ability to Have Full Erections	61.5% (8)	57.1% (8)
Reduction in Ability to Have Erections	15.4% (2)	15.4% (2)	Reduction in Ability to Have Erections	46.2% (6)	57.1% (8)
Function Same as Before RT or Better	84.6% (11)	84.6% (11)	Function Same as Before RT or Better	61.5% (8)	57.1% (8)

Effects of Genistein on CpG Methylation and Histone Acetylation Have Been Reported From Several Groups

Cancer Prevention Clin Cancer Res 2005;11(19) October 1, 2005

Reversal of Hypermethylation and Reactivation of *p16^{INK4a}*, *RARβ*, and *MGMT* Genes by Genistein and Other Isoflavones from Soy

Ming Zhu Fang,[1] Dapeng Chen,[1] Yi Sun,[1] Zhe Jin,[1] Judith K. Christman,[2] and Chung S. Yang[1]

Int. J. Cancer: **123,** 552–560 (2008)

Genistein mediated histone acetylation and demethylation activates tumor suppressor genes in prostate cancer cells

Nobuyuki Kikuno[1], Hiroaki Shiina[2], Shinji Urakami[2], Ken Kawamoto[1], Hiroshi Hirata[1], Yuichiro Tanaka[1], Shahana Majid[1], Mikio Igawa[2] and Rajvir Dahiya[1]*

[1]*Department of Urology, Veterans Affairs Medical Center and University of California, San Francisco, San Francisco, CA*
[2]*Department of Urology, Shimane University School of Medicine, Izumo, Japan*

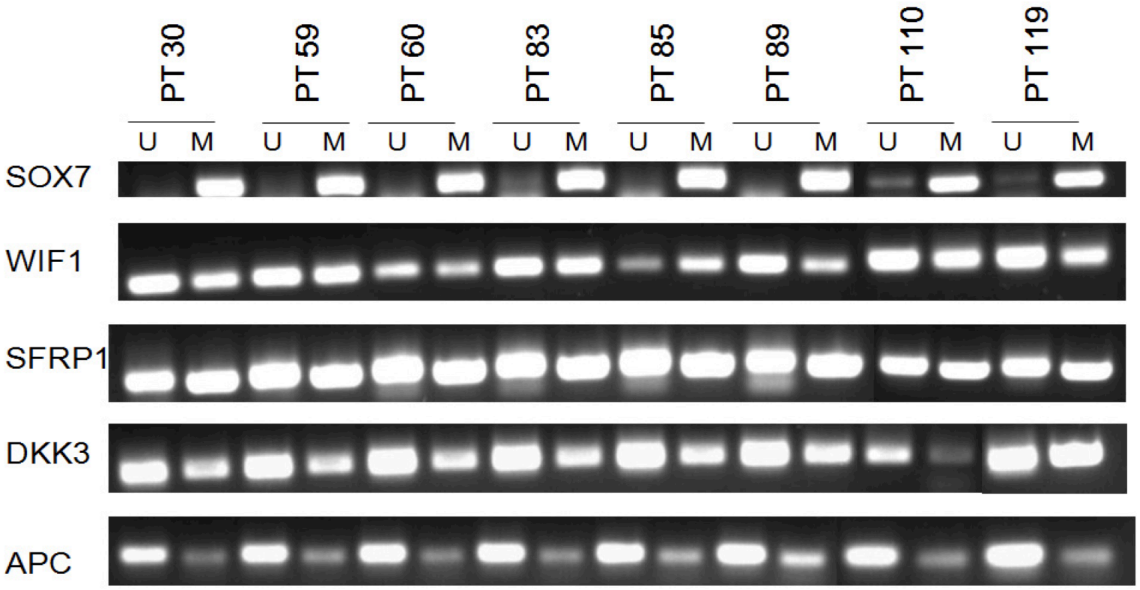
Original Article

Cancer January 1, 2010

Genistein Reverses Hypermethylation and Induces Active Histone Modifications in Tumor Suppressor Gene B-Cell Translocation Gene 3 in Prostate Cancer

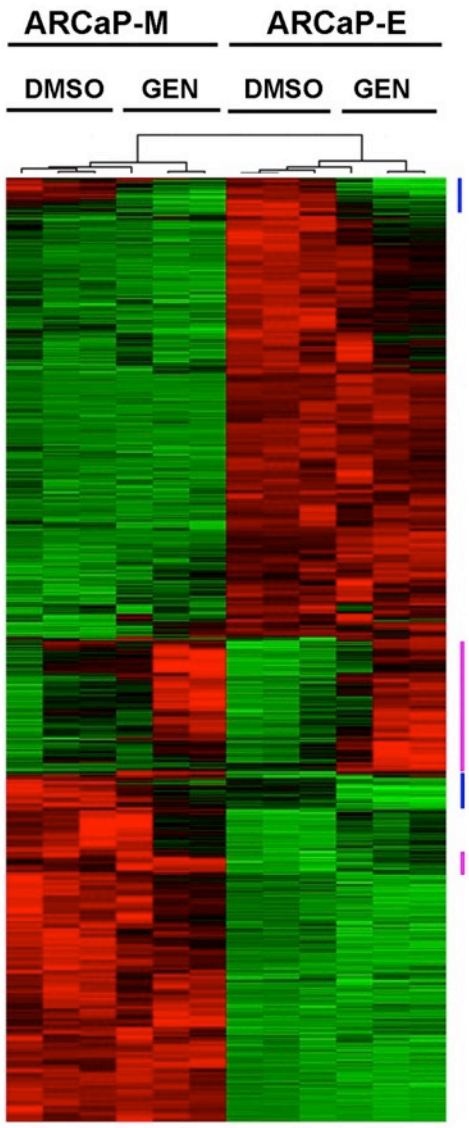
Shahana Majid, PhD[1]; Altaf A. Dar, PhD[2]; Varahram Shahryari, MD[1]; Hiroshi Hirata, MD, PhD[1]; Ardalan Ahmad, MD[1]; Sharanjot Saini, PhD[1]; Yuichiro Tanaka, PhD[1]; Angela V. Dahiya[1]; and Rajvir Dahiya, PhD[1]

Wnt Pathway Inhibitory Genes are hypermethylated in prostate cancer patients



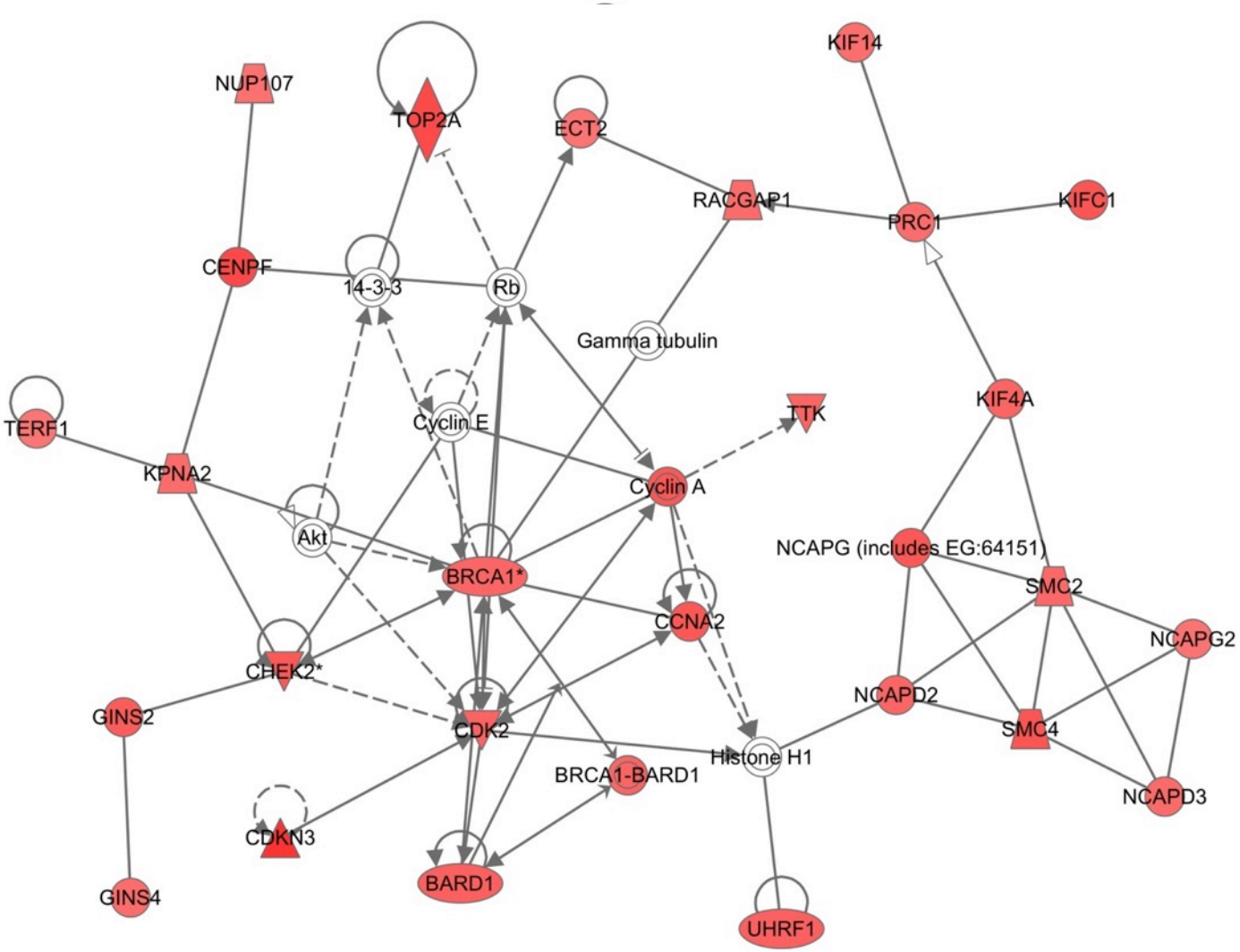
Phillip *et al, BMC Cancer,* 2012, **12(1):**145.

Whole Genome Expression Profiling Of Prostate Cancer Cells Treated with Genistein

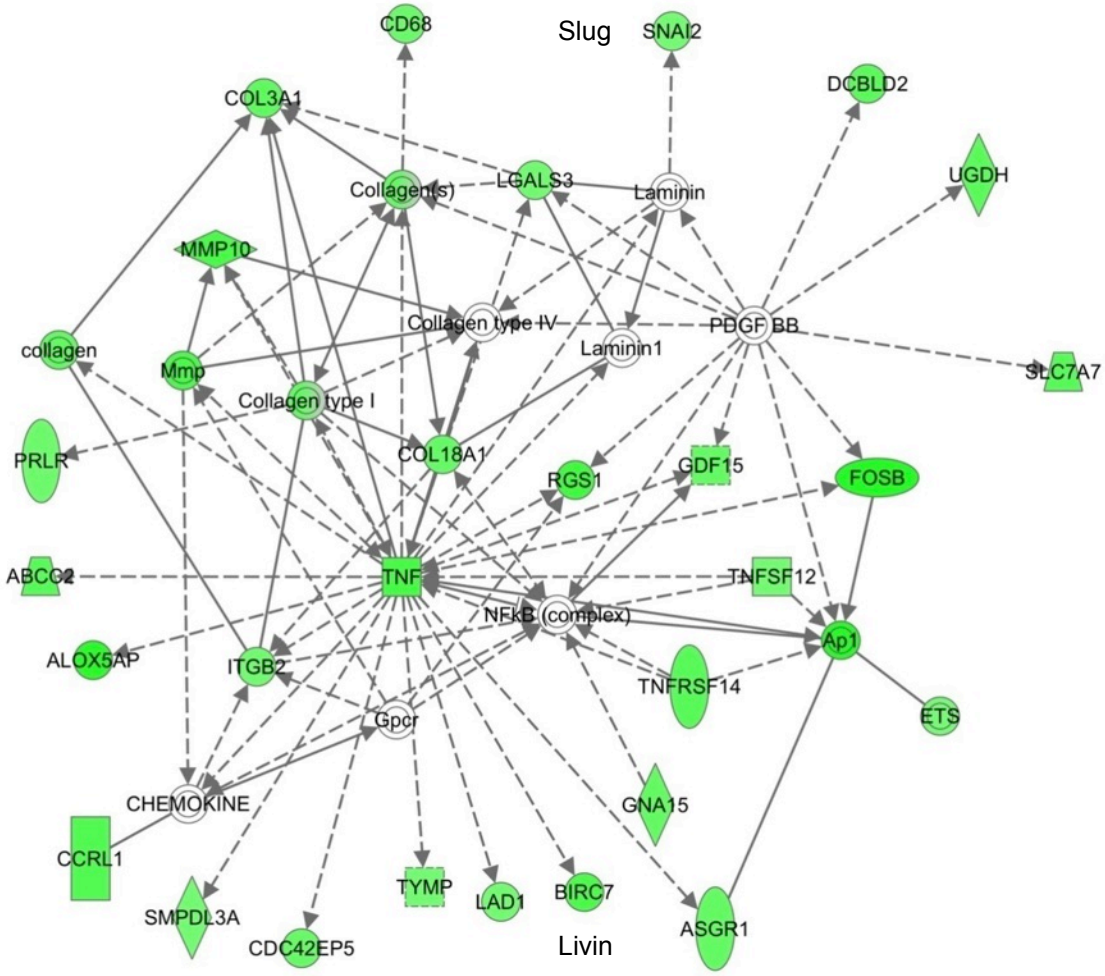


Phillip *et al, BMC Cancer,* 2012, **12(1):**145.

Genistein Upregulates Genes Involved in Cell Cycle Responses to DNA Damage

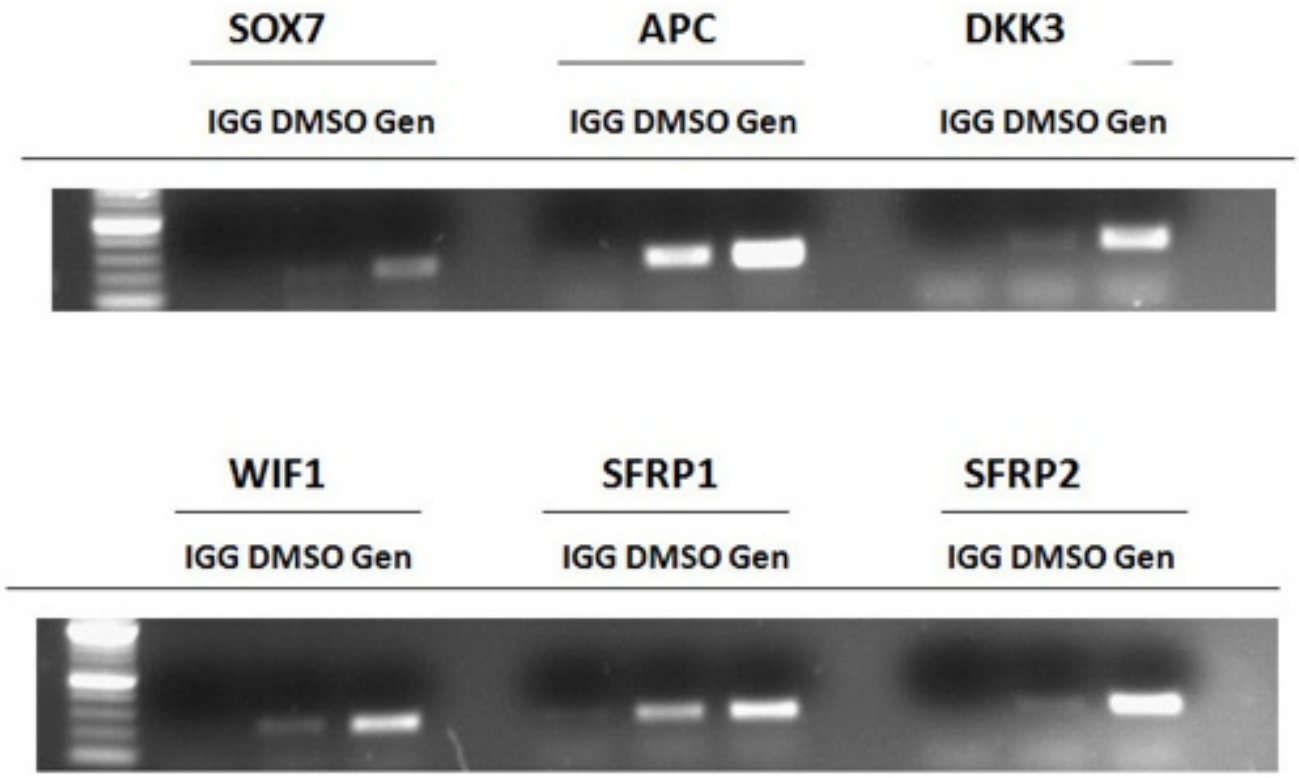


Genistein Downregulates Genes Involved in the TNF-NFKB Pathway



Genistein induces Acetylation of Histone H3K9

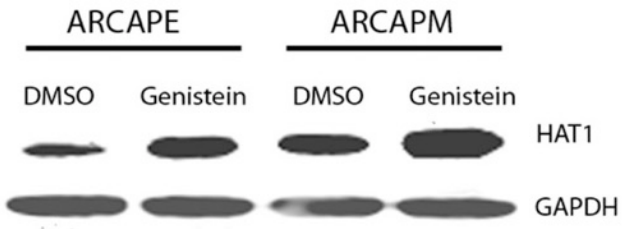
Anti-Ac-H3K9 Chromatin Immunoprecipitation



Phillip *et al, BMC Cancer,* 2012, **12(1):**145.

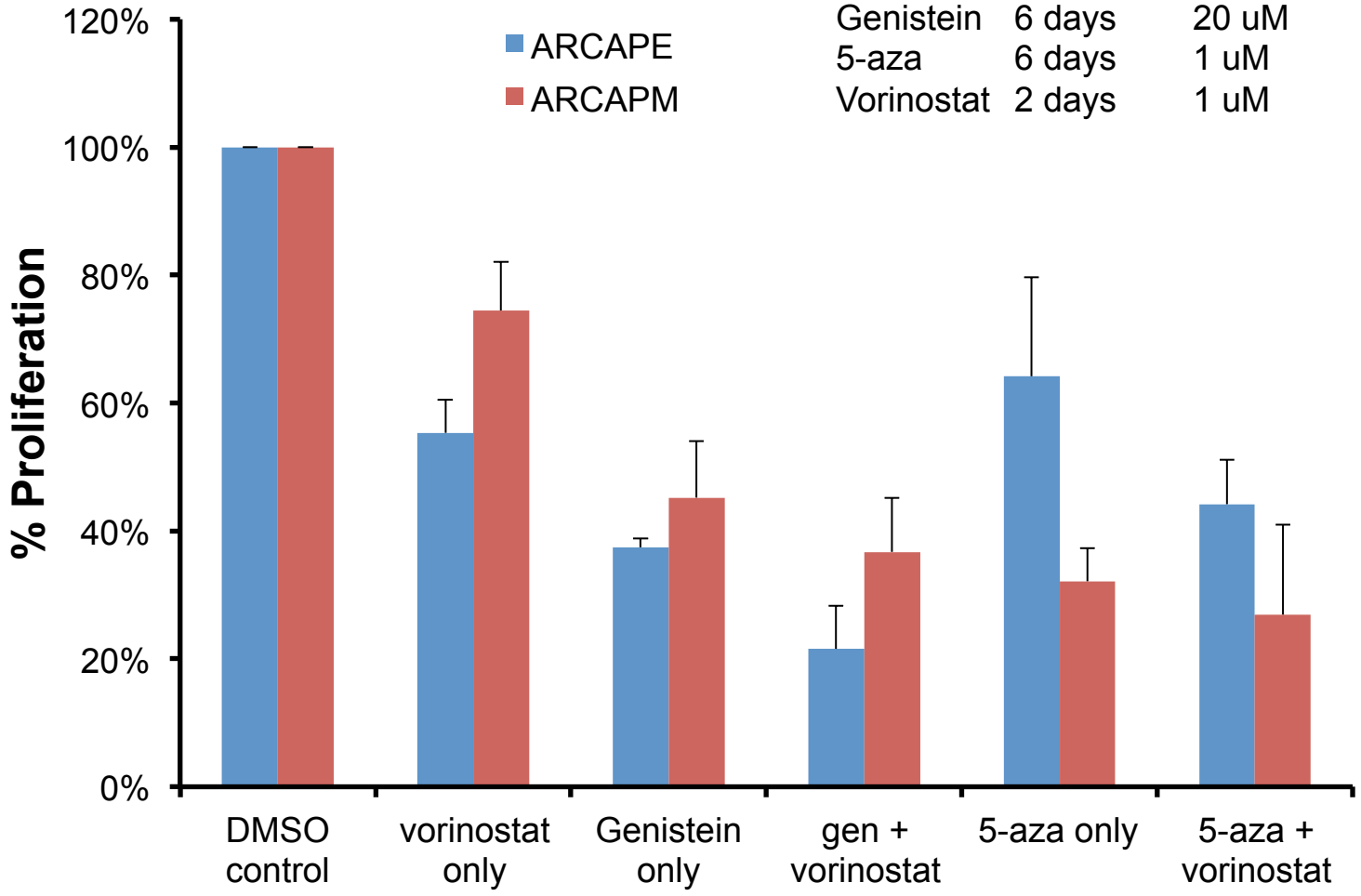
Genistein induces expression of HAT1

Histone Acetyl Transferase 1 (HAT1)



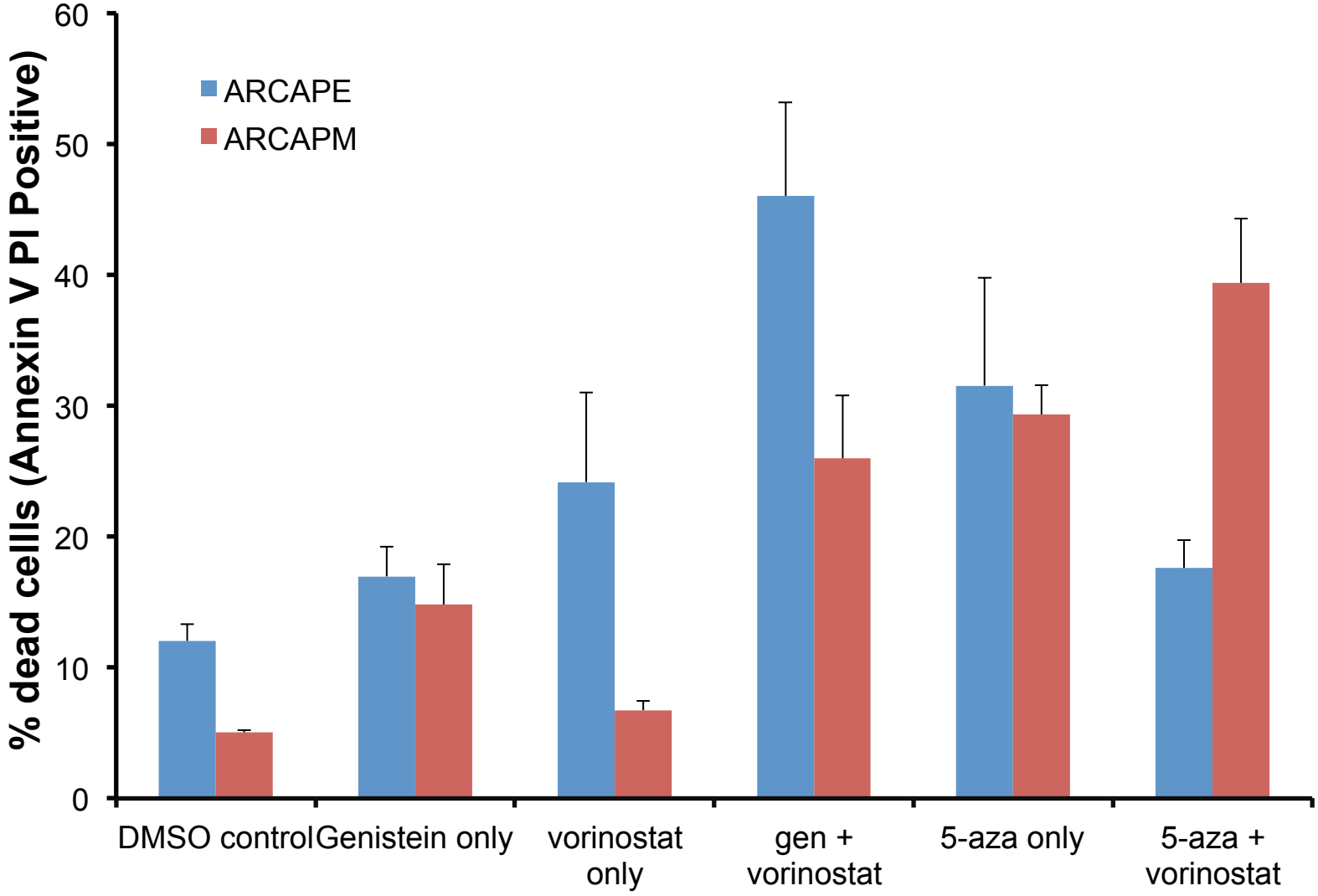
Phillip *et al, BMC Cancer,* 2012, **12(1):**145.

Genistein synergizes with HDACi Vorinostat to inhibit proliferation



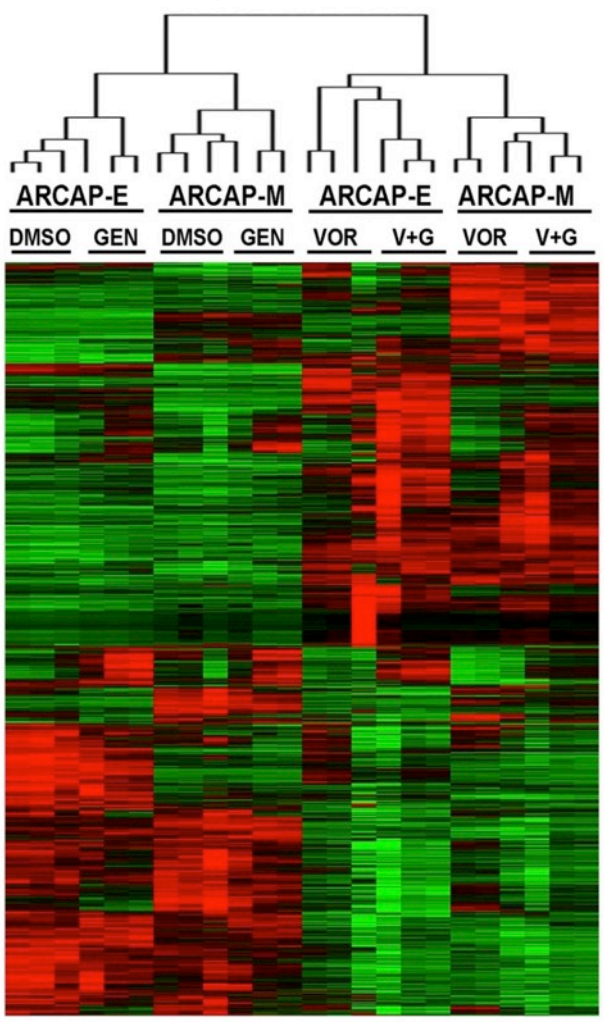
Phillip *et al, BMC Cancer,* 2012, **12(1):**145.

Genistein synergizes with HDACi Vorinostat to induce apoptosis



Phillip *et al, BMC Cancer,* 2012, **12(1):**145.

Whole Genome Expression Profiling Of Prostate Cancer Cells Treated with Genistein, Vorinostat, or Genistein plus Vorinostat



Phillip *et al, BMC Cancer,* 2012, **12(1):**145.

Genistein/Vorinostat Upregulates Genes Involved in Cell Cycle Responses to DNA Damage

GO Term	*Biological Process*	*Count*	*p-value*	*IPA Biological Function*	*Count*	*p-value*
GO:0006281	DNA repair	45	1.03E-13	DNA Replication, Recombination, and Repair	39	7.18E-12
GO:0008219	Cell Death	48	2.73E-03	Cell Death	122	1.95E-10
GO:0022403	Cell Cycle	57	1.63E-14	Cell Cycle	105	2.70E-09
GO:0006915	Apoptosis	43	1.37E-03	Apoptosis	149	1.13E-07
GO:0000075	Cell Cycle Checkpoint	17	1.45E-06	DNA checkpoint control	13	1.90E-06

Phillip *et al, BMC Cancer,* 2012, **12(1):**145.

AMPK and PPAR agonists are exercise mimetics

Ronald M. Evans et al: Cell 134:405-415, 2008

- Natural compounds may mimic or potentiate the effects of exercise and may prevent the development of metabolic syndrome:
  - Natural compounds, such as genistein, have endurance-enhancing activities, but their exact mechanisms remain unclear
- Studied endurance capacities of **mice in a treadmill running test**.
- **PPAR agonist and exercise training synergistically** increased myofibers and running endurance in adult mice.
- **In sedentary mice, 4 weeks of treatment with an AMPK agonist induced metabolic genes and enhanced running endurance by 44%.**

Genistein improves cardiovascular risk factors

Atteritano M et al. Effects of genistein on predictors of cardiovascular risk in osteopenic, postmenopausal women: a two-year randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab. 92:3068-75, 2007.

54 mg genistein + Ca + vitamin D,

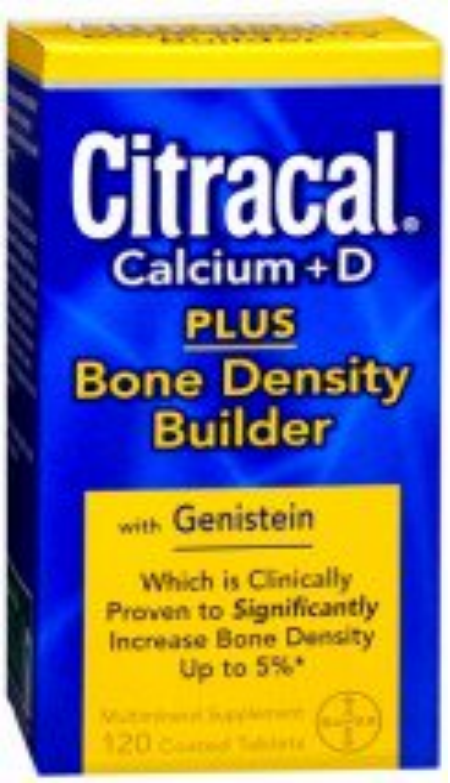
was associated with favorable effects on

glycemic control and

cardiovascular risk markers

Genistein vs Placebo

Citracal plus Genistein



Citracal (Calcium + Vit D)

Genistein, insulin sensitivity and memory

Alonso A et al. Age 2010.

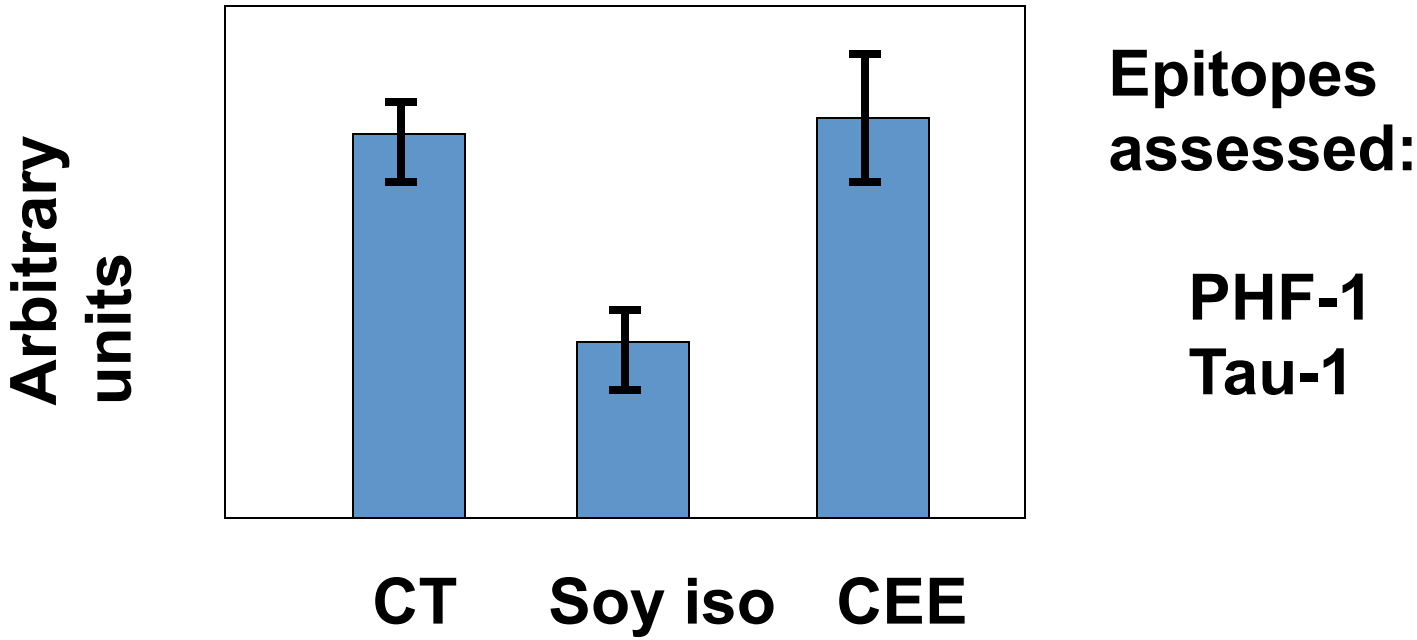
In aged ovariectomized female rats

GENISTEIN

increased insulin sensitivity

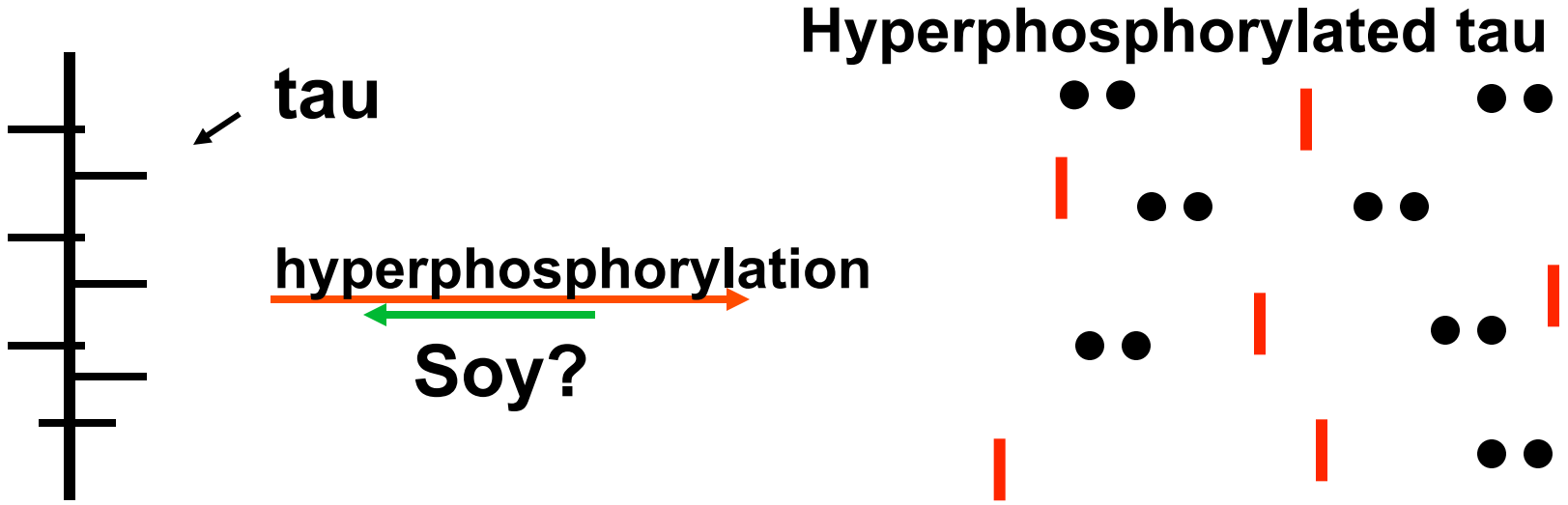
improved spatial memory

Soy isoflavones, but not Premarin, attenuated AD-relevant protein phosphorylation in primate brain.



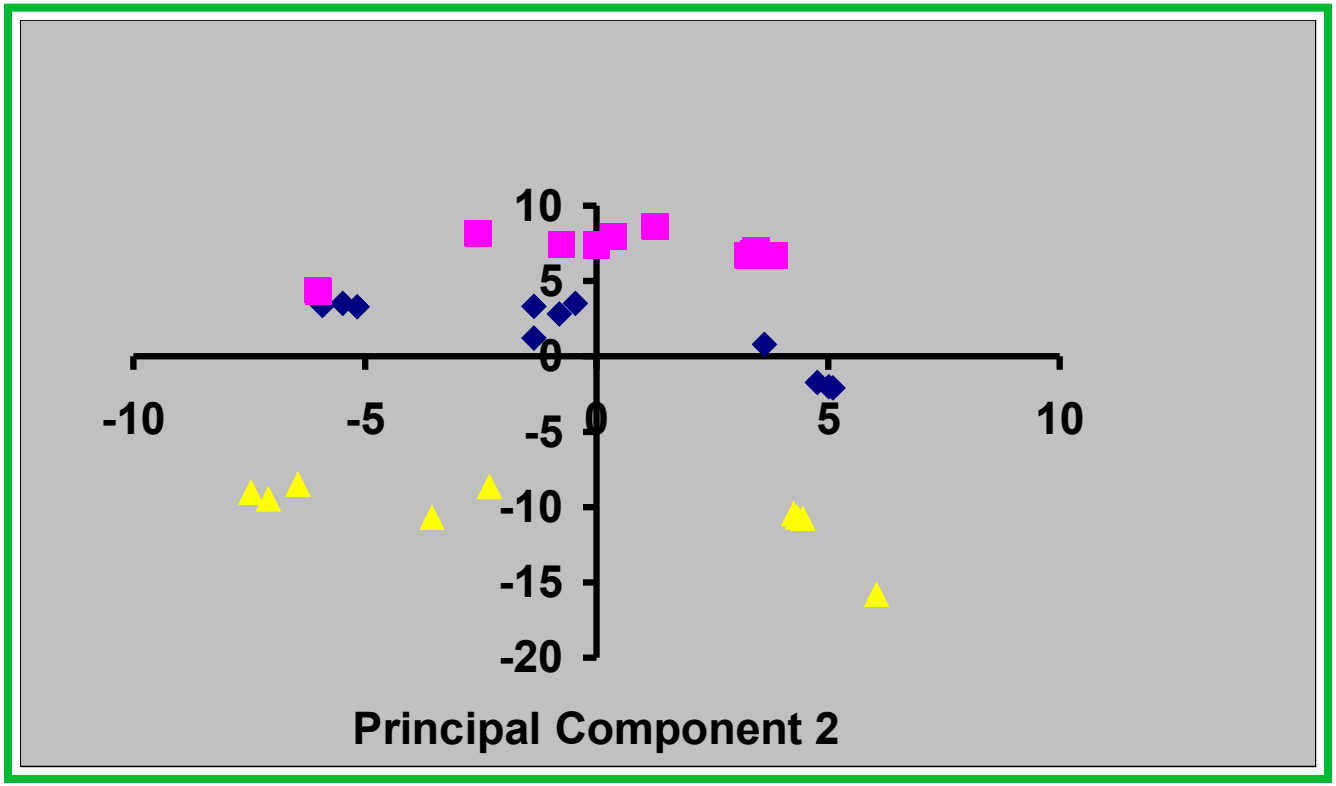
(from H Kim et al., 2001, BioFactors)

Relationship between neuroprotective actions by soy versus estrogen



(Kim, 2001)

Principal Component Analysis indicates that soy+, soy- and casein-based diets, had non-overlapping global effects on brain proteins



casein

soy-

soy+

Isoflavones and cognitive function in older women: the Soy and Postmenopausal Health In Aging (SOPHIA) Study

6-month, double-blind, randomized, placebo-controlled clinical trial

Study subjects were in good health, postmenopausal and not using estrogen replacement therapy

Randomized to active treatment (n = 27) two pills per day, each containing 55 mg of soy-extracted isoflavones (110 mg per day) or placebo (n = 26).

Cognitive function tests administered at baseline and follow-up included: Trails A and B, category fluency, and logical memory and recall (a paragraph recall test assessing immediate and delayed verbal memory).

Kritz-Silverstein D; Von Muhlen D; Barrett-Connor E; Bressel M. **Menopause. 10:196-202, 2003.**

Isoflavones and cognitive function in older women: Soy & Postmenopausal Health In Aging (SOPHIA) Study.

At baseline, all women were cognitively intact; there were no significant differences by treatment assignment in age, education, depressed mood, or cognitive function (all P values > 0.10).

The women in the treatment group did consistently better, both as compared with their own baseline scores and as compared with the placebo group responses at 6 months.

Comparisons of percentage change in cognitive function between baseline and follow-up showed greater improvement in category fluency for women on active treatment as compared with the case of those on placebo (P = 0.02) and showed greater improvement on the other tests of verbal memory and Trails B.

Soy isoflavones ameliorate the adverse effects of chemotherapy in children

Tacyildiz N, Ozyoruk D, Yavuz G, Unal E, Dincaslan H, Dogu F, Sahin K, Kucuk O. Nutr Cancer. 2010;62(7):1001-5.

- 9 cycles of chemotherapy were administered without genistein, and 57 cycles with genistein (8 mg/day).
- Patients had less myelosuppression, mucositis, and infection when they received their chemotherapy with genistein.
- During supplementation, serum genistein levels were 2-6 times higher compared to presupplementation levels.
- Patients who received abdominal radiation reported less pain and diarrhea when they took the genistein supplement.

Summary

- Genistein
  - Antioxidant (prevents DNA damage)
  - Anti-inflammatory (IL-1, IL-6 inhibition)
  - DNA demethylation
  - Histone acetylation
  - NFkB, RANKL, VEGF, MMP, EMT inhibition
  - Enhances chemo/RT
  - Reduces toxicities of chemo/RT
  - Potentiates immune function (anti-viral, anti-bacterial)

Genistein in survivorship research

- Opportunities for *prevention of short term and long term adverse effects* of radiation and chemotherapy:
  - » Second primary tumors
  - » Cognitive decline
  - » Cardiac toxicity
  - » Myelosuppression
  - » Pulmonary toxicity
  - » Neurotoxicity (CNS and peripheral neuropathy)
  - » Nephrotoxicity
  - » Hepatotoxicity
- Improved efficacy of chemo/RT and targeted therapy
- Genistein is a safe, orally bioavailable compound which has been well tolerated in clinical trials

Let food be your medicine.

Hippocrates

okucuk@emory.edu