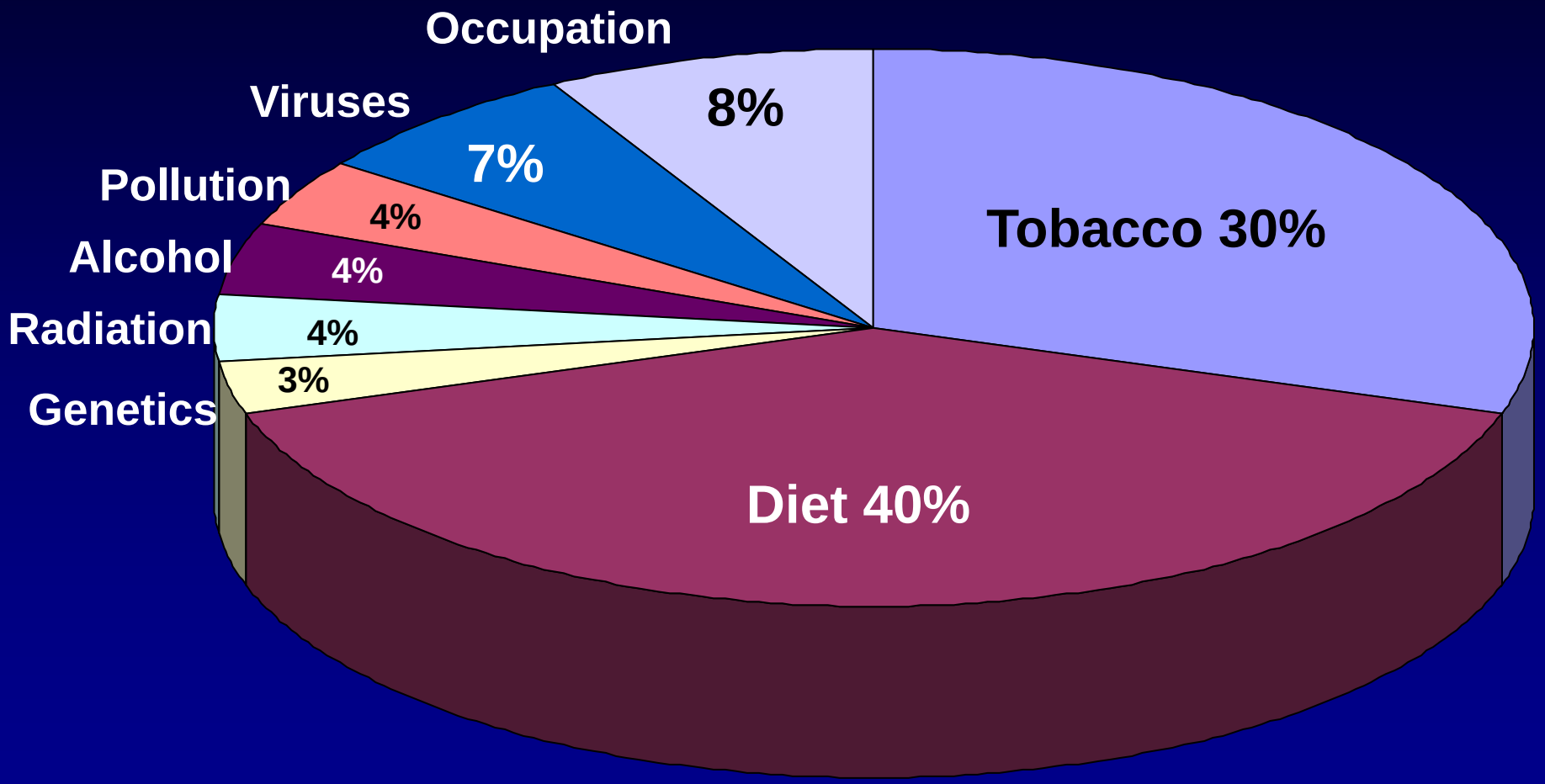
Cancer Prevention with Berries

Gary Stoner, PhD
Professor Emeritus
Department of Medicine
Ohio State University

Factors Causing Cancer



Approaches to Cancer Prevention

- Identify individuals at high risk of acquiring cancer
- Reduce exposure to environmental carcinogens
- Chemoprevention
  - The prevention of cancer development by the use of dietary and synthetic chemicals

Estimates of Cancer Deaths Avoidable by Dietary Change

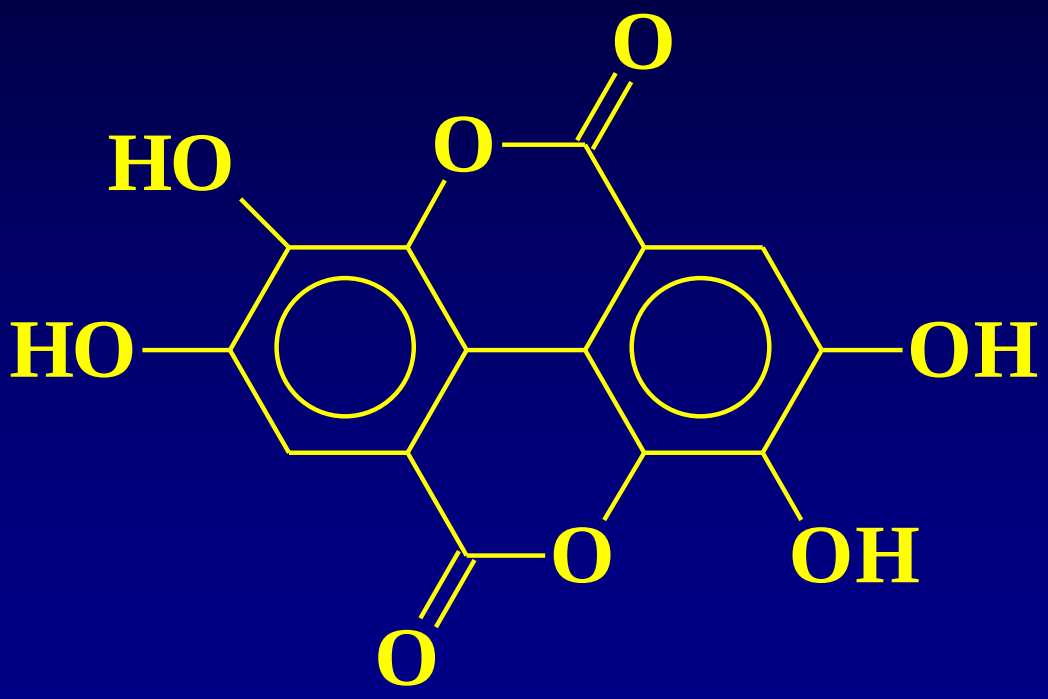
	Percent Avoidable	
	Doll-Peto	Willett
Type	(1981)	(1984)
Lung	20	20
Colon/rectum	90	70
Breast	50	50
Prostate	50	75
Pancreas	50	50
Stomach	35	35
Endometrium	50	50



Naturally-occuring cancer preventative (chemopreventive) agents

Agent	Source
diallyl sulfide	garlic, onion
sulforaphane	broccoli
resveratrol	grapes
EGCG	green tea
lycopene	tomatoes
limonene	oranges, limes
curcumin	tumeric
phenethyl isothiocyanate	various cruciferiae
vitamins: A,C,E, folic acid	numerous sources

Ellagic Acid (phenol)



Concentration of Ellagic Acid in Fruits

Fruit	μg EA/gm dry weight
Black Raspberries	1548 ± 142
Red Raspberries	1473 ± 108
Strawberries	630 ± 90
Cranberries	120 ± 4
Apples	< 100
Blueberries	< 100
Blue Plums	< 100
Cherries (sour, bing)	< 100
Elderberry	< 100
Grapes (white, red)	< 100
Grapefruit (white, pink)	< 100
Oranges (navel)	< 100
Peaches	< 100

E Daniel, et al., *J. Food Comp Anal*. 2:338, 1989

Black Raspberries (BRBs)



Freeze-dried powder



Method for Preparing and Standardizing Berry Powder

Black raspberries

↓

Pick mechanically and wash thoroughly

↓

Freeze on farm (2-3 hrs)

↓

Freeze dry and grind into powder

↓

Test powder for:

Concentrations of 26 nutrients and non-nutritive constituents

Contamination with herbicides, fungicides, and pesticides

Contamination with *E. coli, Salmonella, Listeria* and fungi

Potential Cancer Preventive Agents in Black Raspberries

- Vitamins: A, C, E, folic acid
- Minerals: calcium, selenium (potassium)
- Simple phenols: ellagic acid, ferulic acid, coumaric acid, quercetin
- Complex phenols: anthocyanins
- Phytosterols: β-sitosterol, campesterol, stigmasterol
- Fiber: pectin and other complex polysaccarides

L Kresty, et al., *Cancer Res*. 61:6112, 2001; G. Stoner, et al., *Cancer Prev. Res*. 2:187, 2009

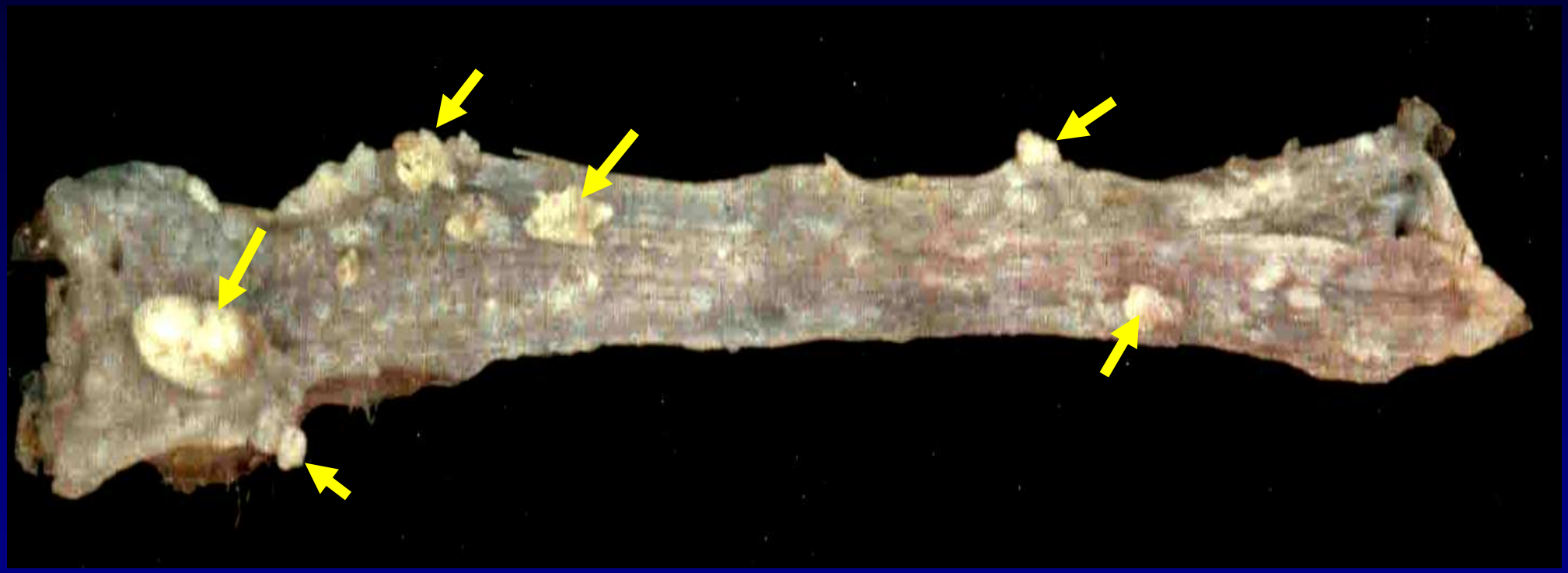
Nine month toxicity study



Gastrointestinal Tract Cancers

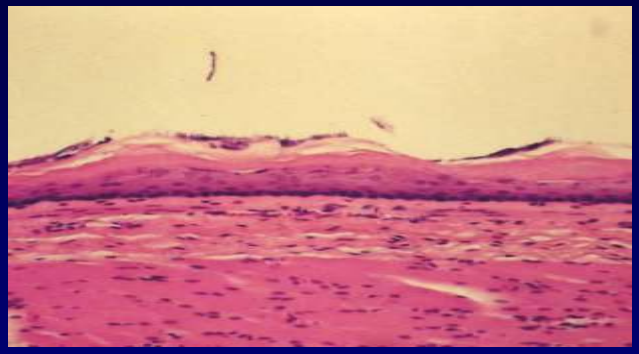
- Esophagus
  - Sixth most common cancer worldwide
  - Squamous cell carcinoma – 90%
  - Adenocarcinoma – most common in U.S.
  - 5-year survival = 6-7%
- Colon
  - Second major cause of cancer death in U.S.
  - Adenocarcinoma
  - 5-year survival = 64%

Gross Lesions in NMBA-treated Rat Esophagus

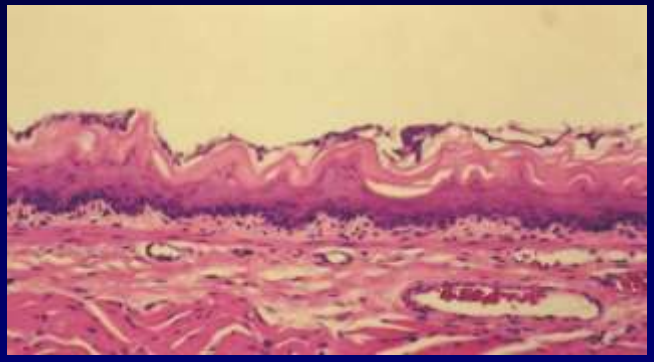


NMBA-INDUCED LESIONS IN RAT ESOPHAGUS

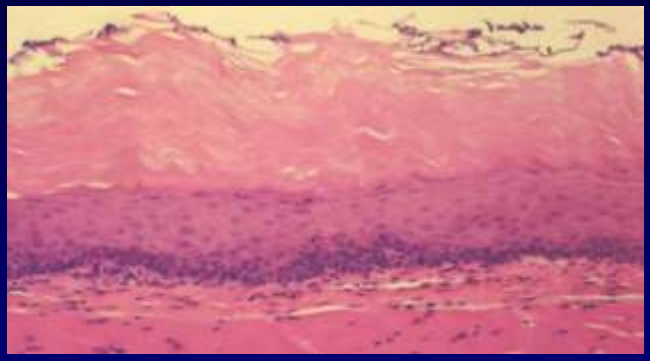
Normal



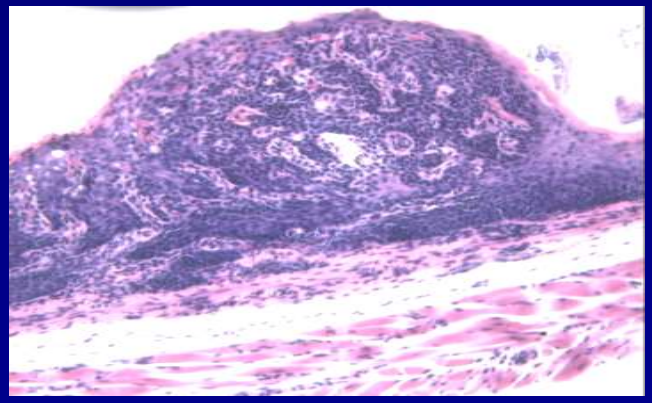
Hyperplasia



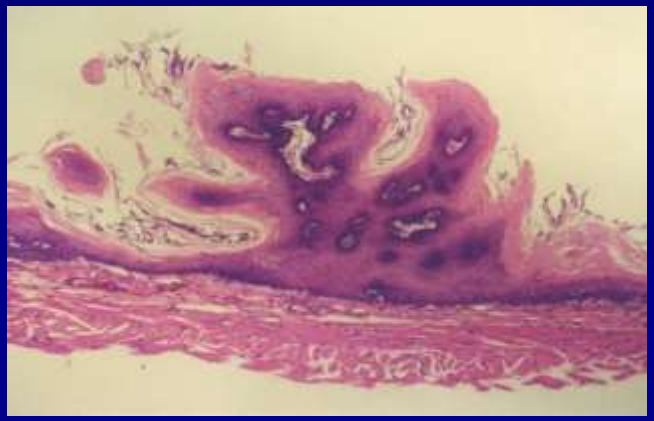
Leukoplakia



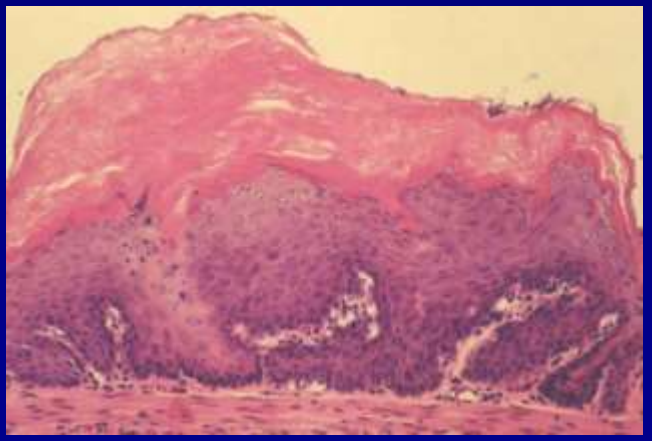
Squamous Cell Carcinoma



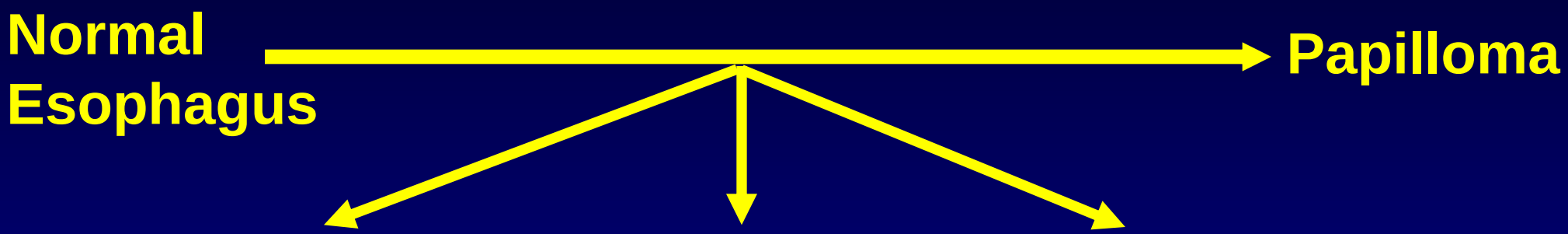
Papilloma



Dysplasia



Molecular Events Associated with Esophageal Papilloma Development



H-ras (~90%)

p53 (~30%)

p27, p16 (n.c.)

PCNA ↑

TGF-α, EGFR ↑

cyclins D_1, E ↑

COX-1, COX-2 ↑

TGF-β receptor ↓

iNOS and ODC ↑

VEGF-1 ↑, HIF1-α

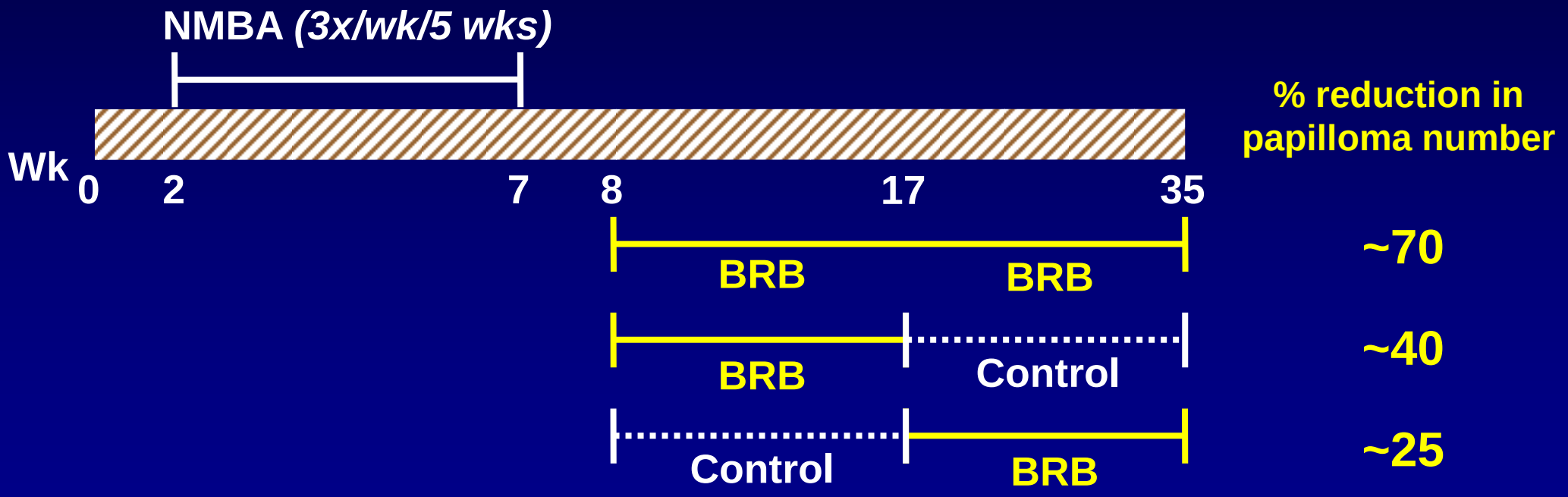
α6, β4 integrin ↓

E-cadherin, α-catenin (localization)

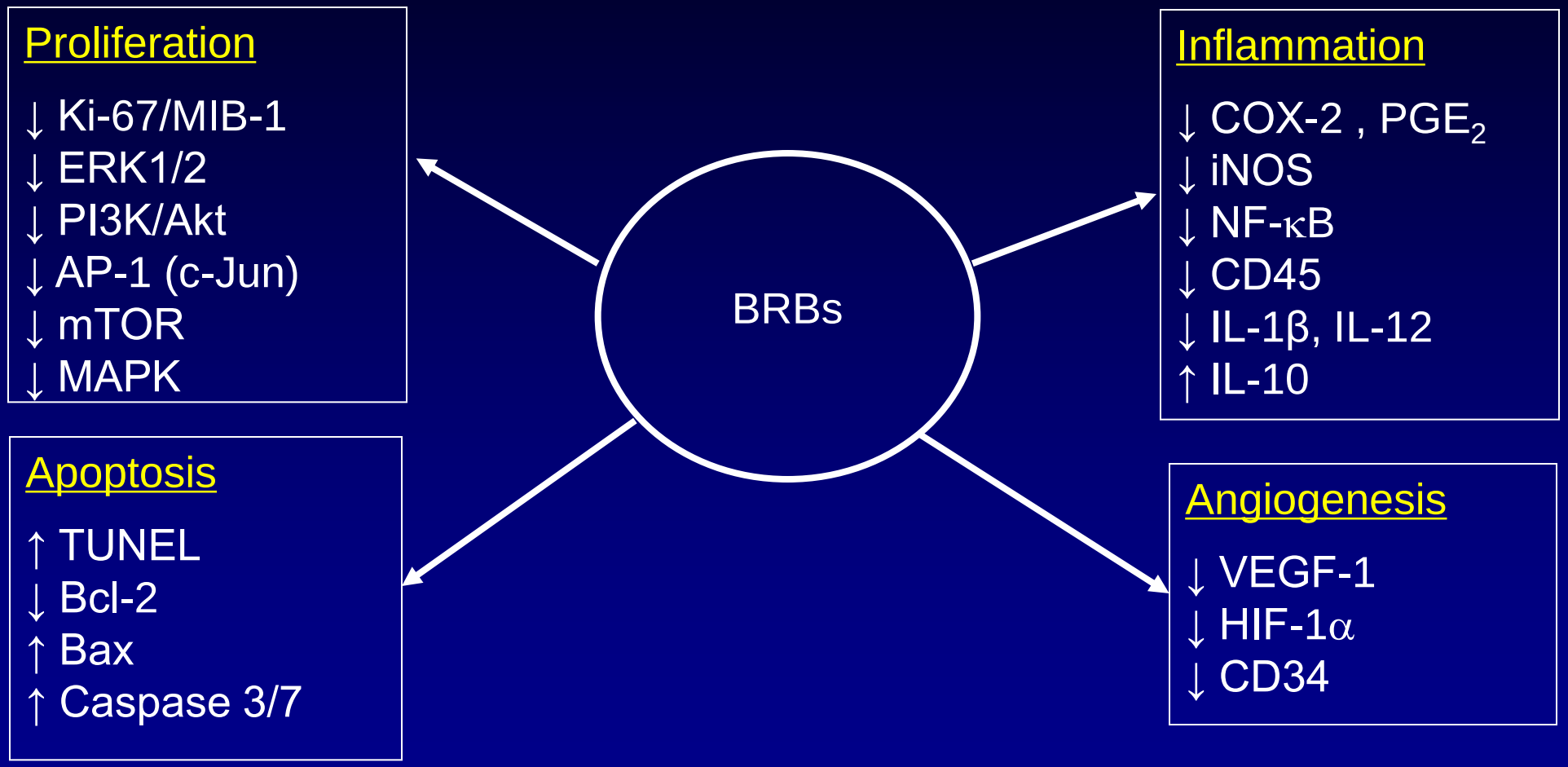
Matrix metalloproteinases ↑

G. Stoner and A. Gupta, Carcinogenesis 22:1737, 2001

Inhibition of Esophageal Tumor Number by 5% Black Raspberry (BRB) Diet in the Post-initiation Bioassay



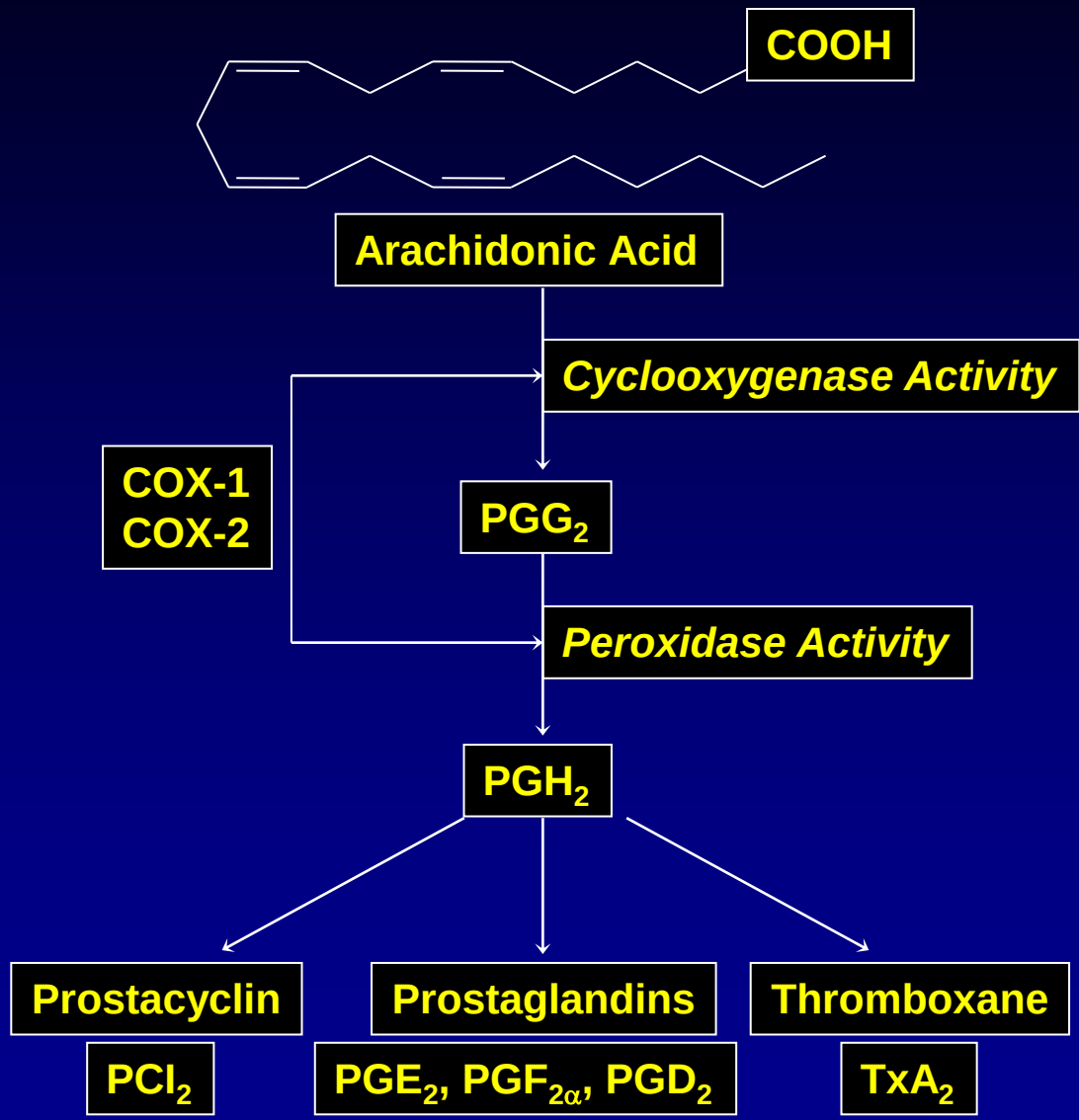
Effects of BRBs on Cellular Events and Associated Genes in NMBA-Treated Rat Esophagus



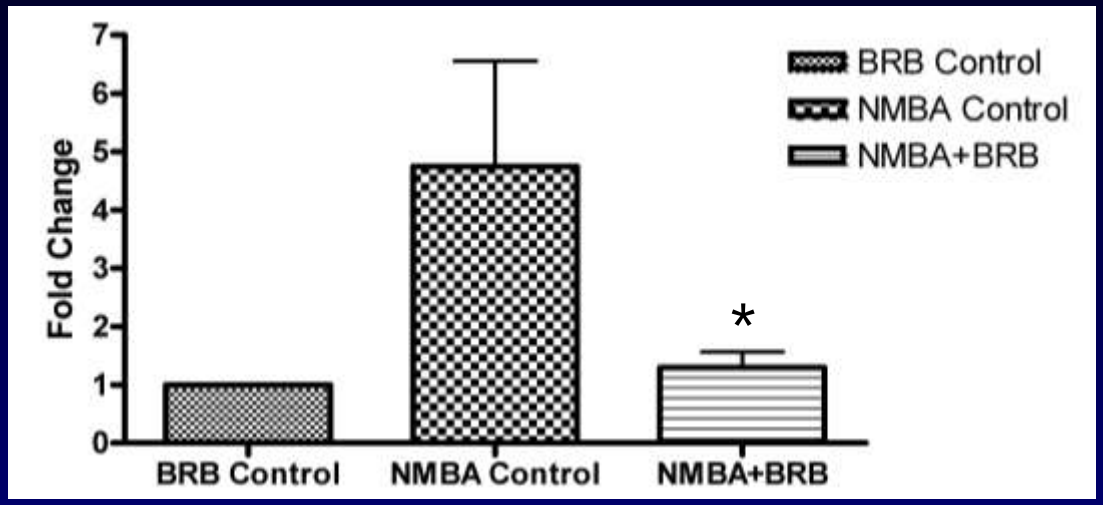
CD45 – leukocyte common antigen, CD34 – Microvessel marker

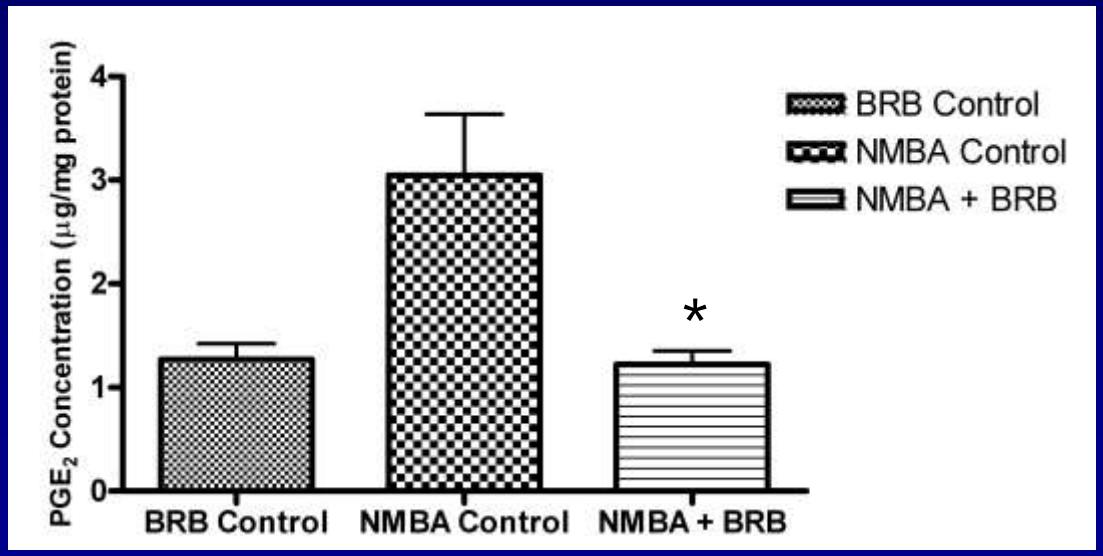
L-S. Wang et al., Cancer Prev Res. 2009;2:84.

Arachidonic Acid Metabolism



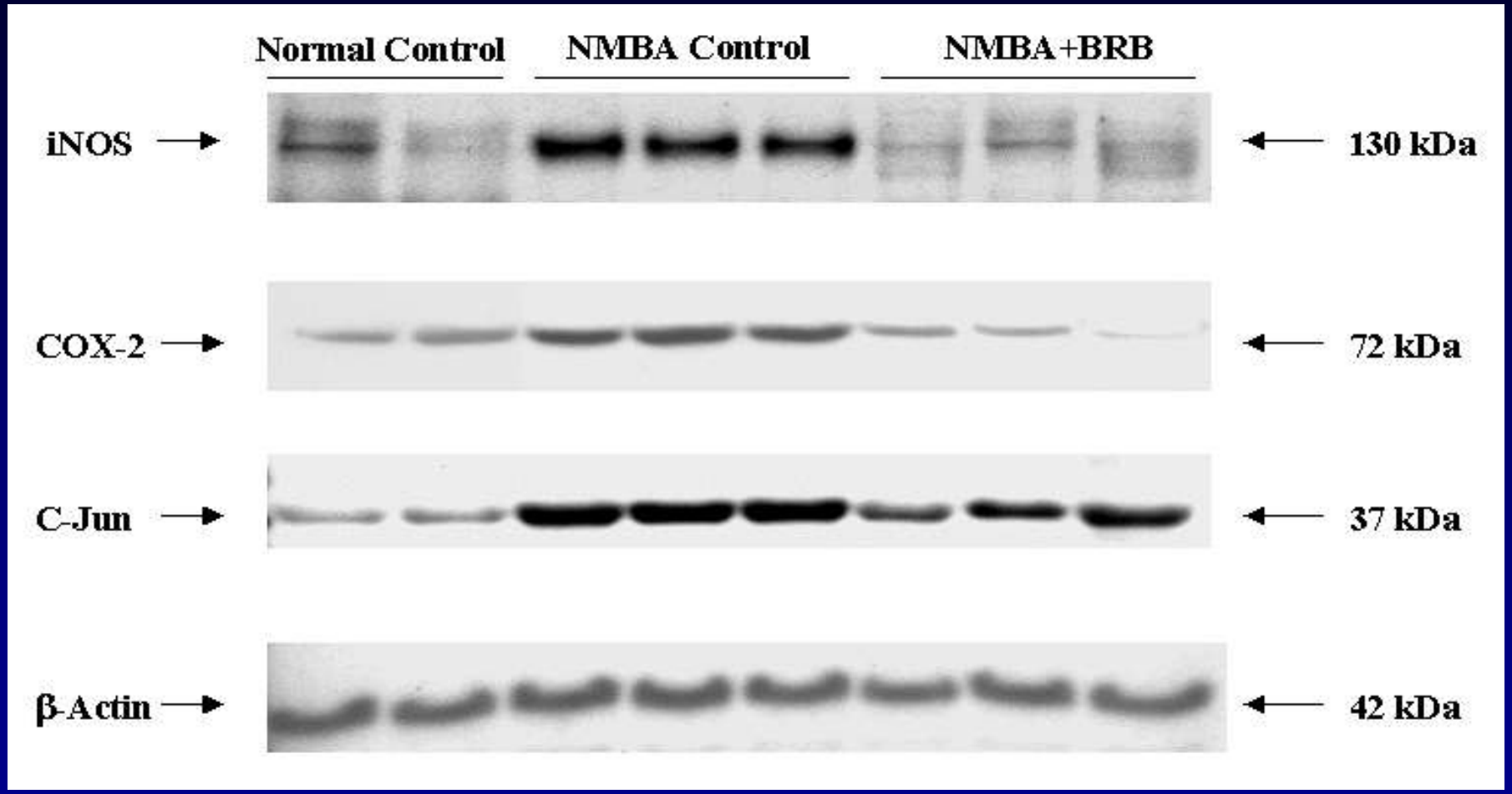
Effect of Black Raspberries on COX-2 Expression and Activity in NMBA-Treated Rat Esophagus



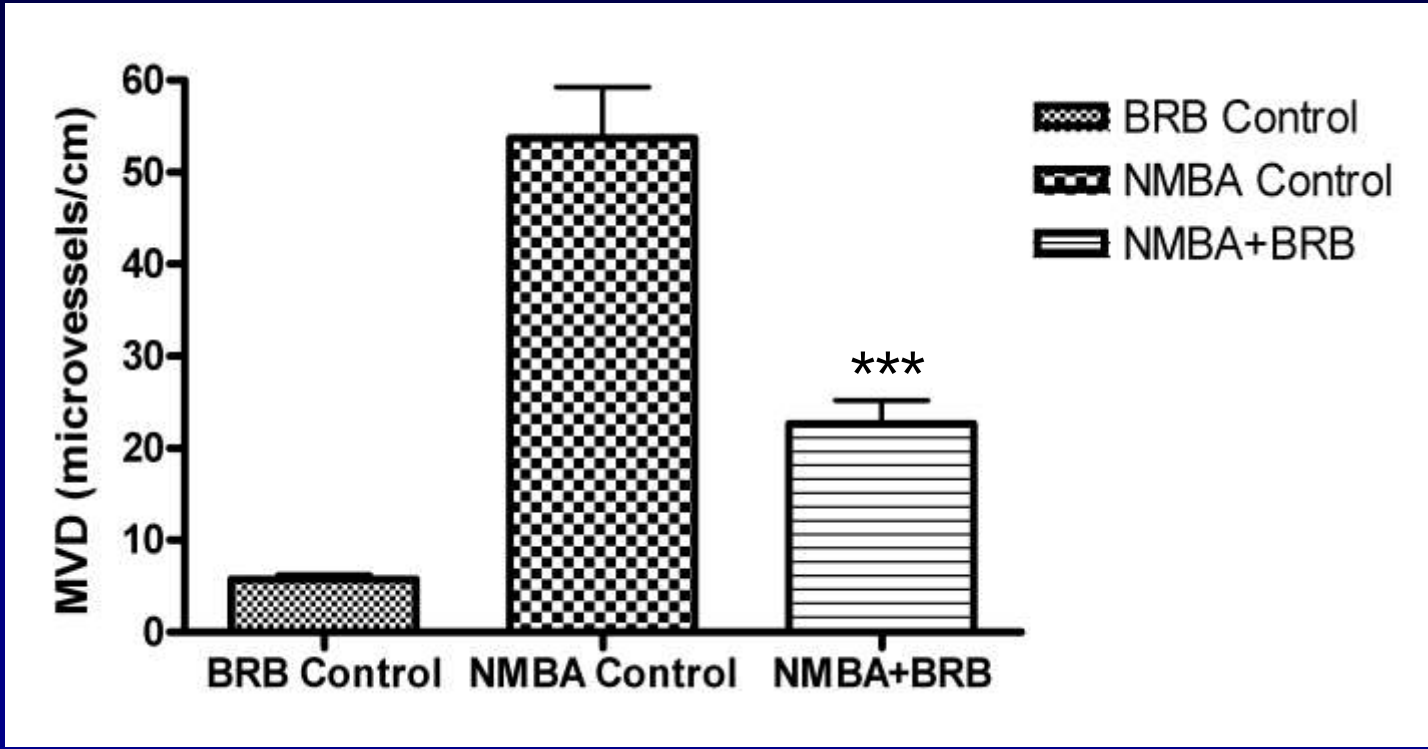


Group

Western Blot Analysis of iNOS, COX-2 and c-Jun

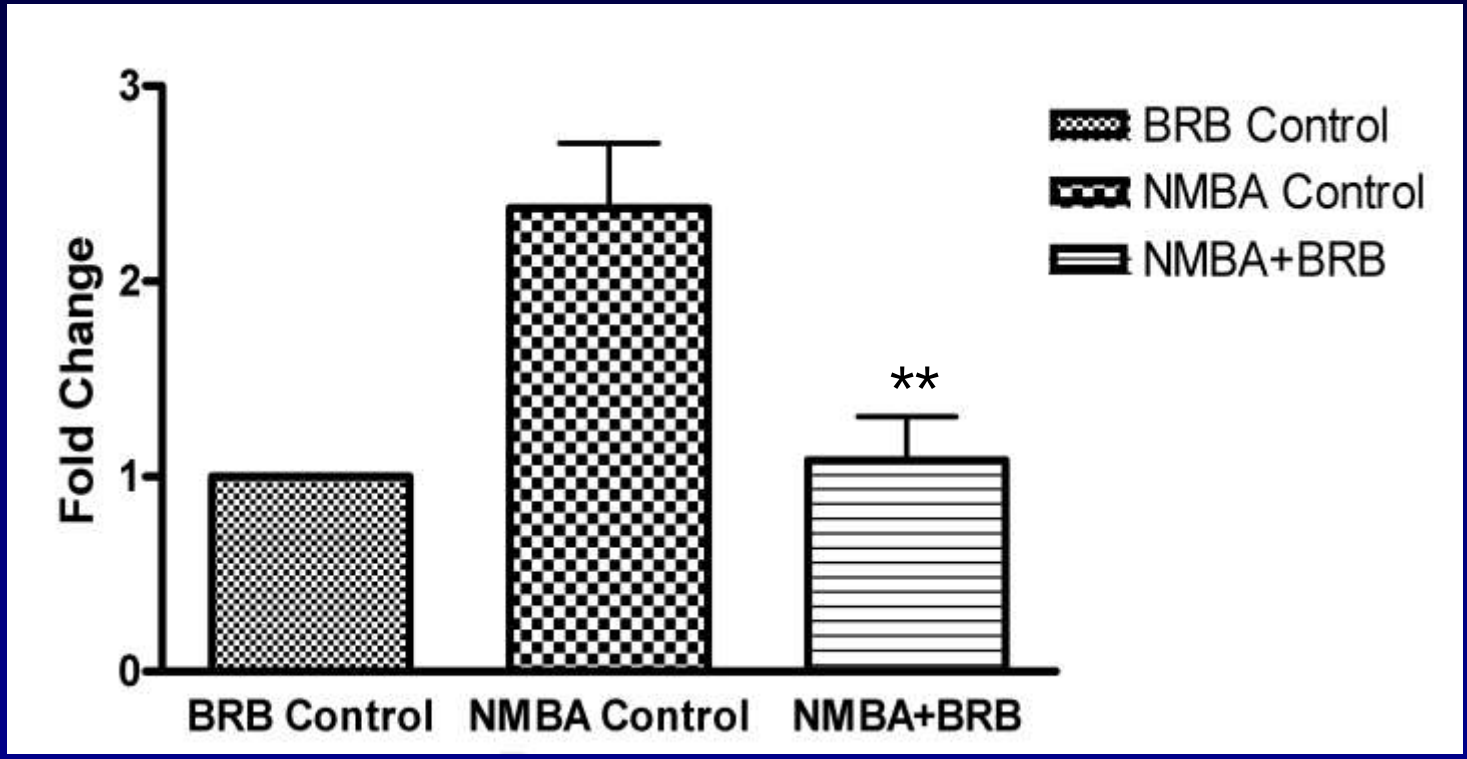


Effect of Black Raspberries on Microvessel Density (MVD) in NMBA-Treated Rat Esophagus



*** $P < 0.0005$

Effect of Black Raspberries on VEGF-1 Expression in NMBA-Treated Rat Esophagus



** *P* < 0.005

Mechanisms of Esophagus Cancer Prevention in Rats with Black Raspberries

Berries:

- **Reduce growth rate of premalignant cells**
- **Reduce inflammation**
- **Inhibit new blood vessel formation**
- **Stimulate apoptosis (programmed cell death)**
- **Promote cell adhesion and cell-cell communication**
- **Inhibit tissue invasion**

LA Kresty et al., *Cancer Res*. 61:6112,2001; T Chen et al., *Cancer Res*. 66:2853,2006; T Chen et al., *Carcinogenesis* 27:2301,2006; G. Stoner, et al. *Cancer Res.* 68:6460, 2008; L-S Wang et al., *Cancer Prev. Res*. 2:84-93, 2009.

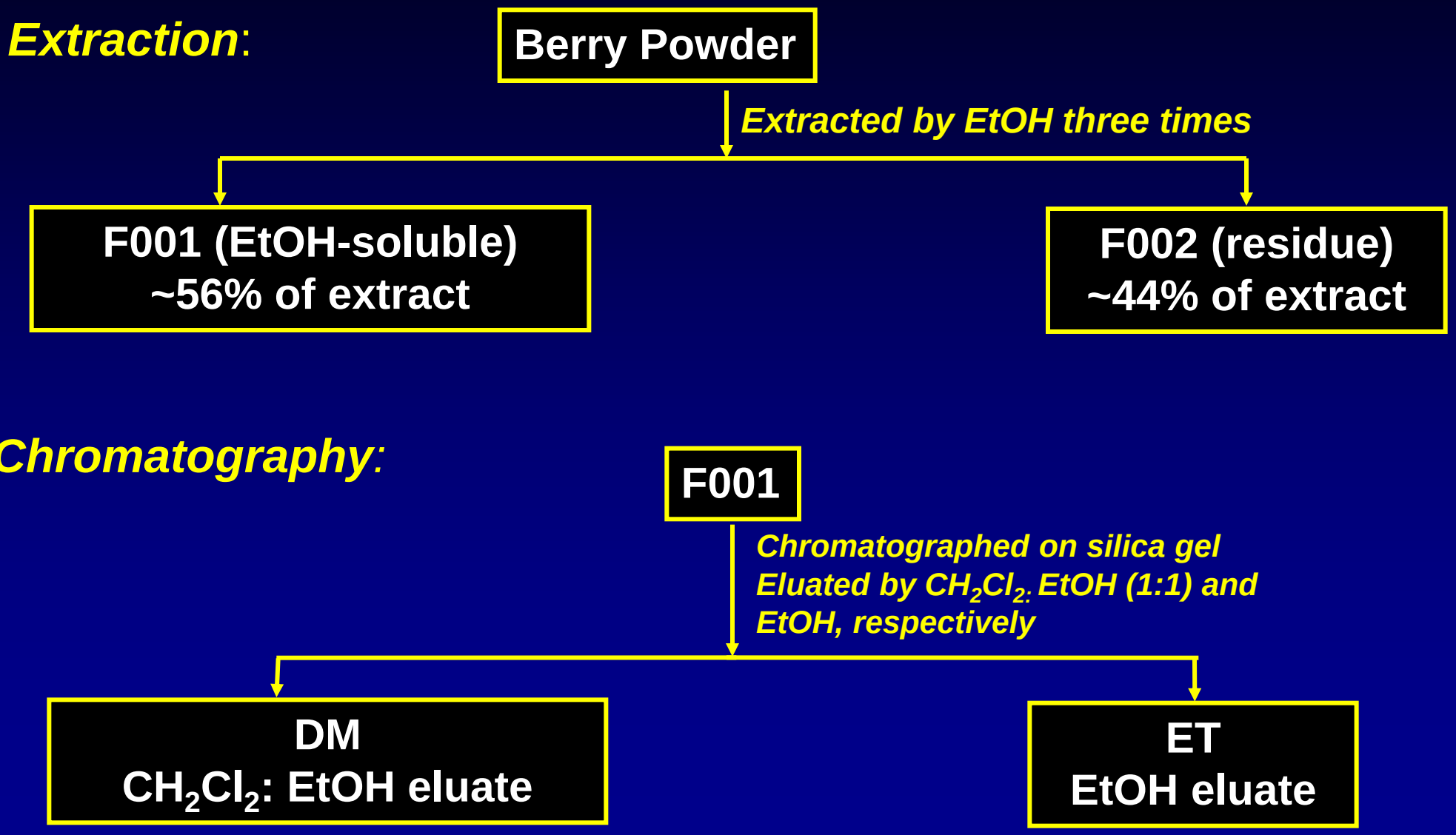
BLACK RASPBERRIES – ANIMAL MODELS

Species	Site	Carcinogen	Berries	% Tumor Reduction
hamster	cheek pouch	[a]DMBA	5 & 10% in diet	8 – 56
rat	mammary gland	estrogen	1 & 2.5% in diet	43-51
rat	esophagus	[a]NMBA	5 & 10% in diet	43 – 68
rat	colon	[a]AOM	2.5, 5.0 & 10% in diet	42 – 71
IL-10 mouse	colon	colitis	10% in diet	46 – 57
SKH-1 mouse	skin	[a]UVB	500 µg extract	77

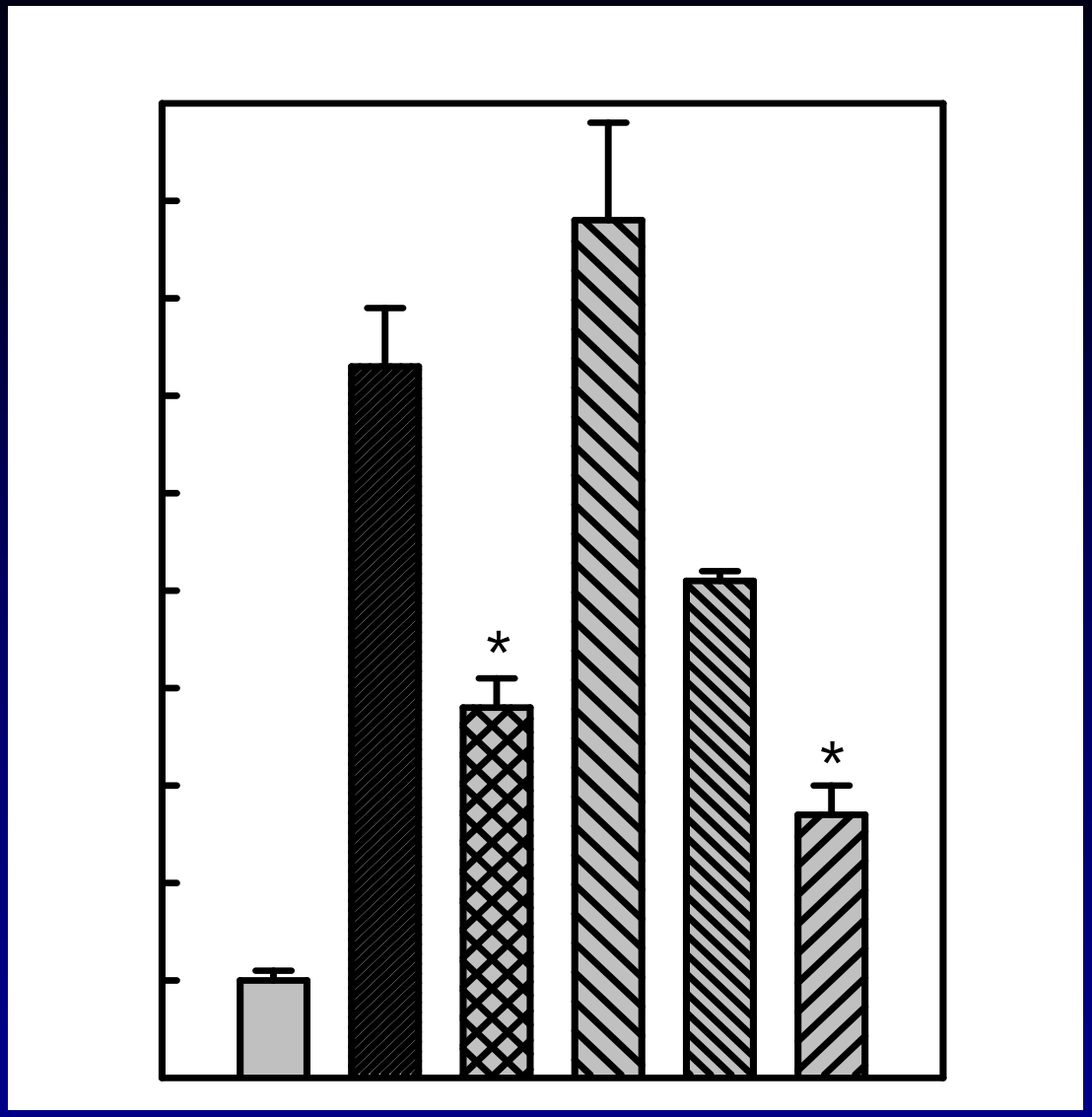
[1]Casto *et al.*, Anticancer Res. 22: 4005, 2002 ; [2] Aiyer *et al*. Nutr. Cancer 60: 227, 2008; [3]Kresty *et al.*, Cancer Res. 61: 6112, 2001; [4]Wang *et al.,Cancer Prev. Res. 2:183 2009;* [5]*Harris et al*., Nutri. Cancer 40: 125, 2002; [6]Bi *et al.*, Cancer Prev. Res. 3:1443, 2010; [7] Yang *et al.*, Berries and Cancer Prev., Chap. 12, 2011; [8]Duncan *et al.*, Cancer Prev. Res. 2:665, 2009

What are the bioactive compounds in black raspberries?

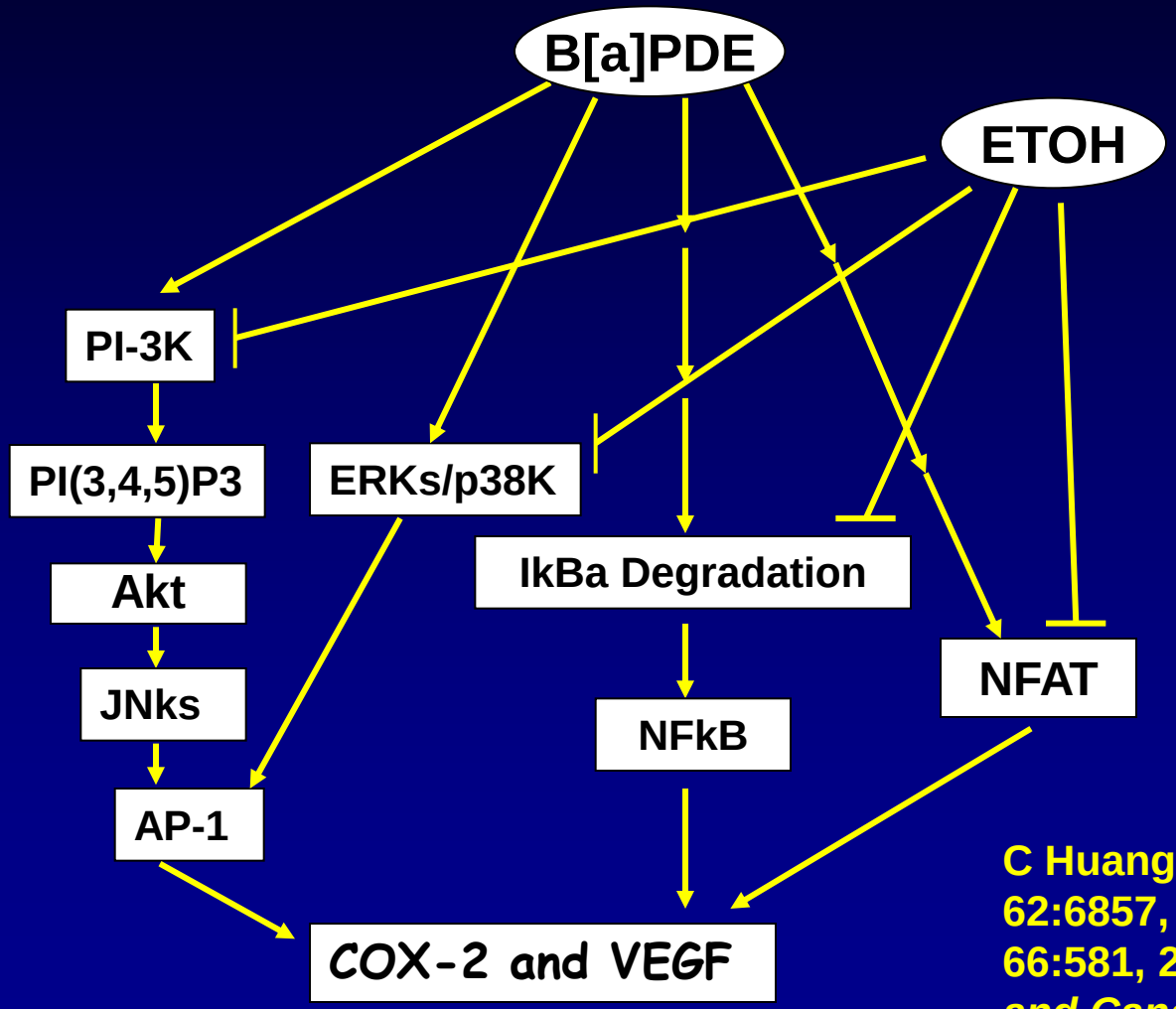
Scheme for Extraction of Freeze-Dried Black Raspberries



Cl 41 cells

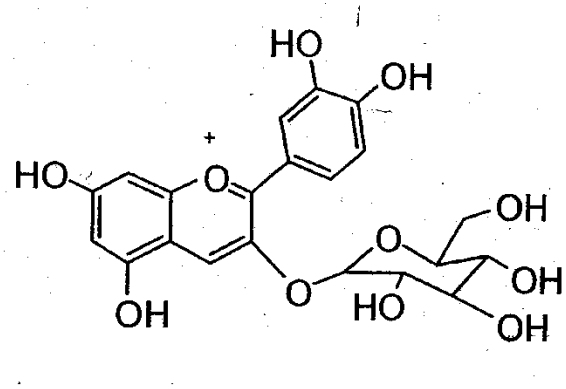

Relative NFκB Activity
*
*
BPDE (2 μM) – + + + + +
Berry Extract RU - (25 μg/ml) – – F003 F004 DM ET

Inhibition of Signaling Pathways by Ethanol Extract (ETOH)

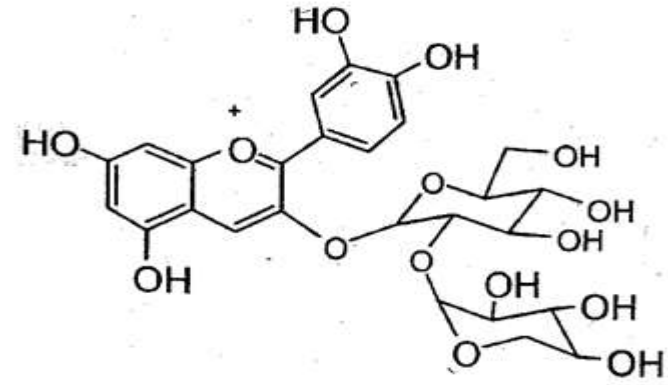


C Huang et al., *Cancer Res.* 62:6857, 2002; *Cancer Res.* 66:581, 2006; H Lu et al., *Nutrition and Cancer* 54:69, 2006

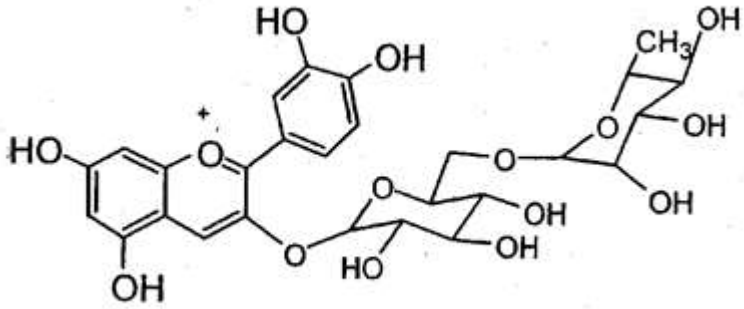
Anthocyanins in Black Raspberries



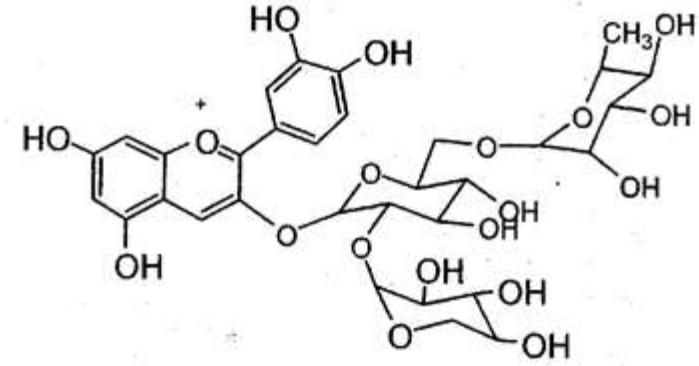
Cyanidin 3-*O*-glucoside



Cyanidin 3-*O*-sambubioside



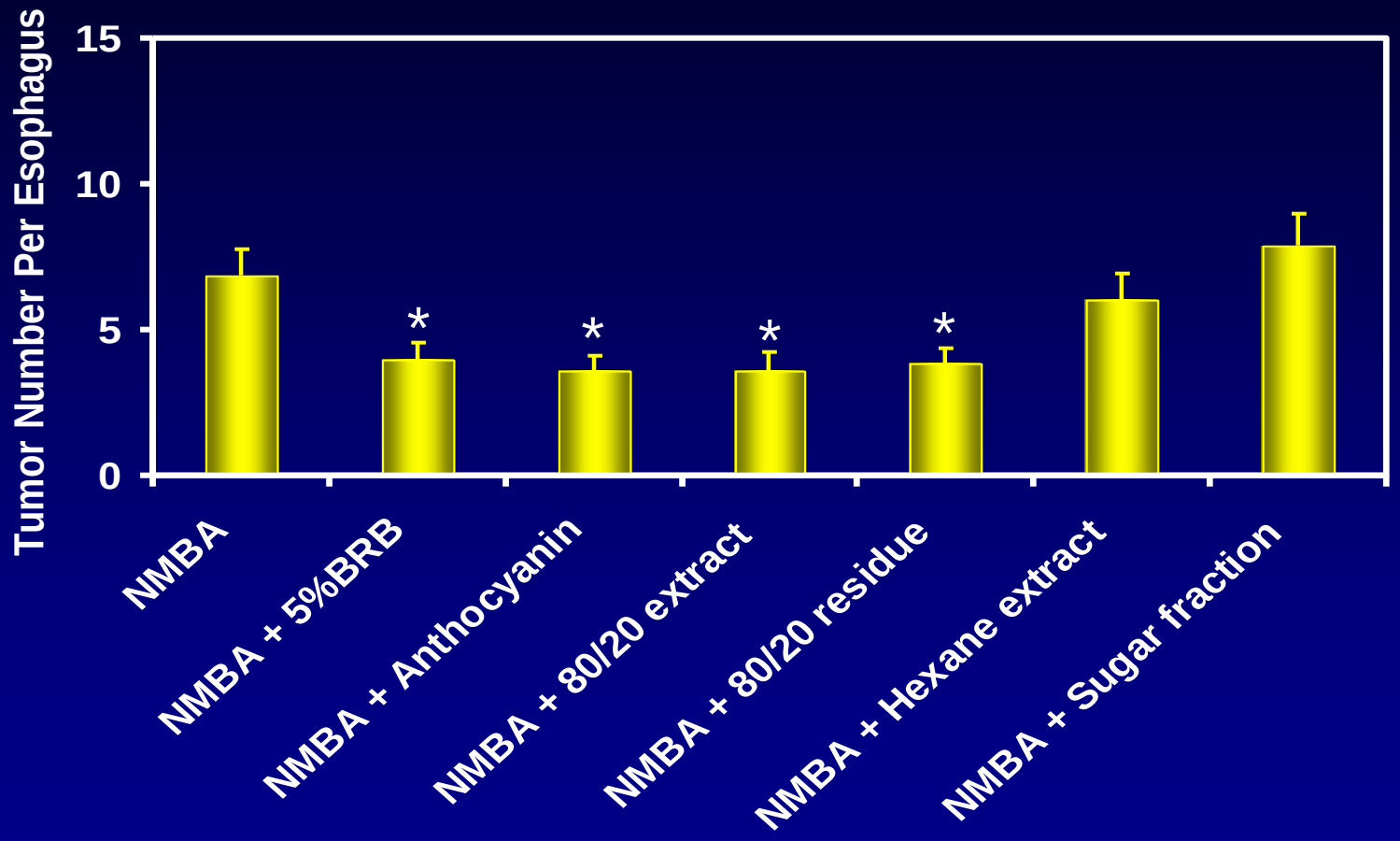
Cyanidin 3-*O*-rutinoside



Cyanidin 3-*O*-(2^{G}-xylosylrutinoside)

SS Hecht et al., *Carcinogenesis* 27:1617, 2006

Effects of BRB Extract on Tumor Development in NMBA-Treated Rat Esophagus at 30 Weeks



Bars: Mean ± SE

N=15

*p<0.05

(v.s. NMBA only)

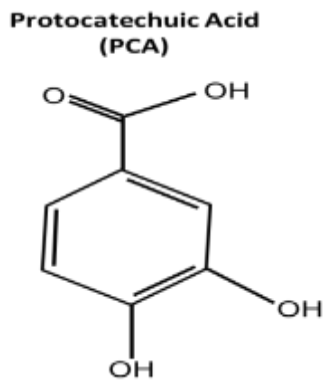
LS Wang et al., Cancer Prev. Res. 2:84-93, 2009

Summary of Bioactive Fractionation Studies

- The anthocyanins in black raspberries appear to be the most active inhibitory constituents.
- However, the insoluble residue fraction is also inhibitory and studies are underway to identify the active components in this fraction.

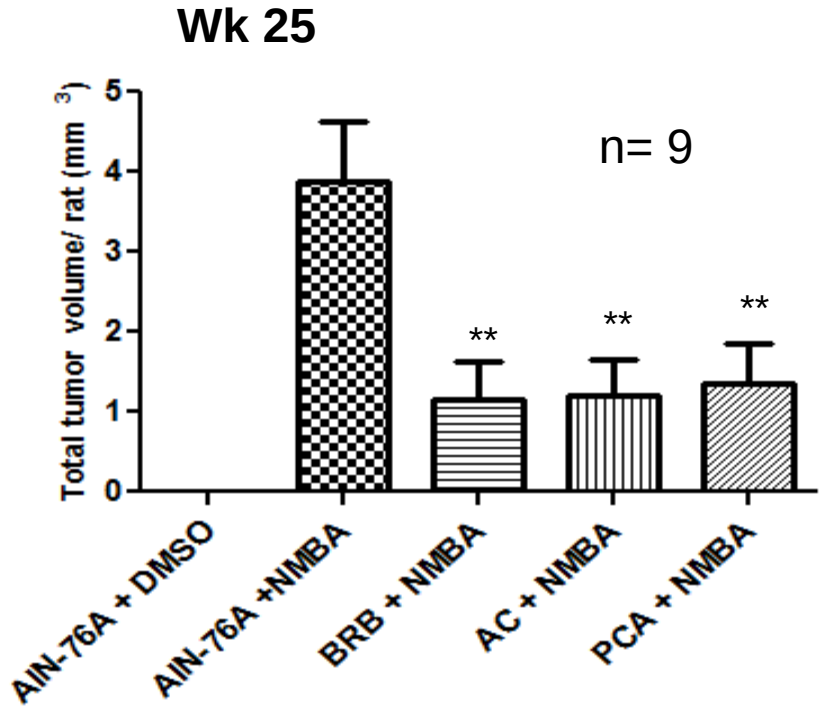
LS Wang et al., Cancer Prev. Res. 2:84-93, 2009

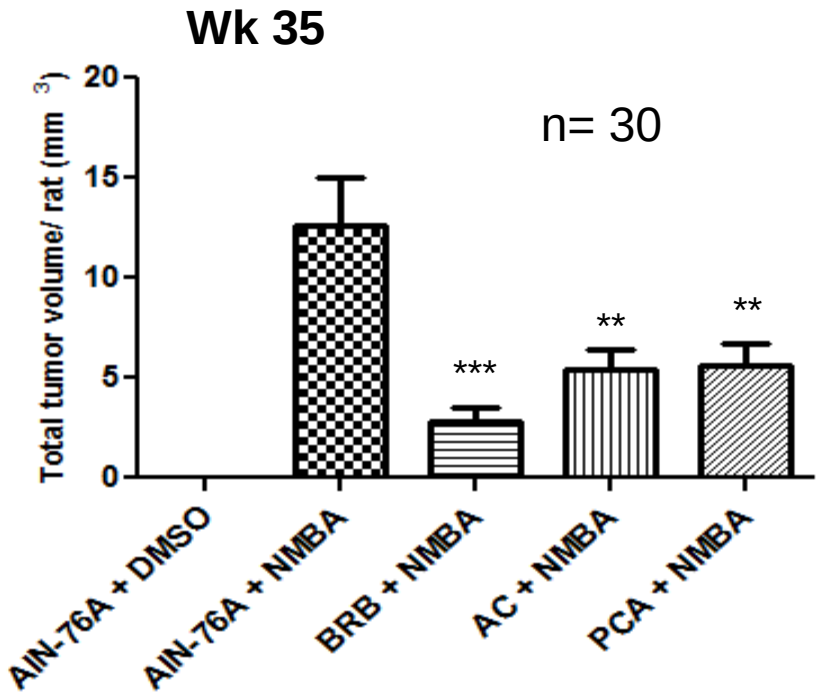
Protocatechuic acid (PCA)



- Simple phenolic acid
- Major stabile microbial-mediated metabolite of cyanidin-type anthocyanins
- Antioxidant, antiproliferative and pro-apoptotic
- Favorable toxicity profile
- Cancer chemopreventive: rat colon, bladder, mouse lung

Effects of BRB, AC and PCA on Esophageal Tumor Burden



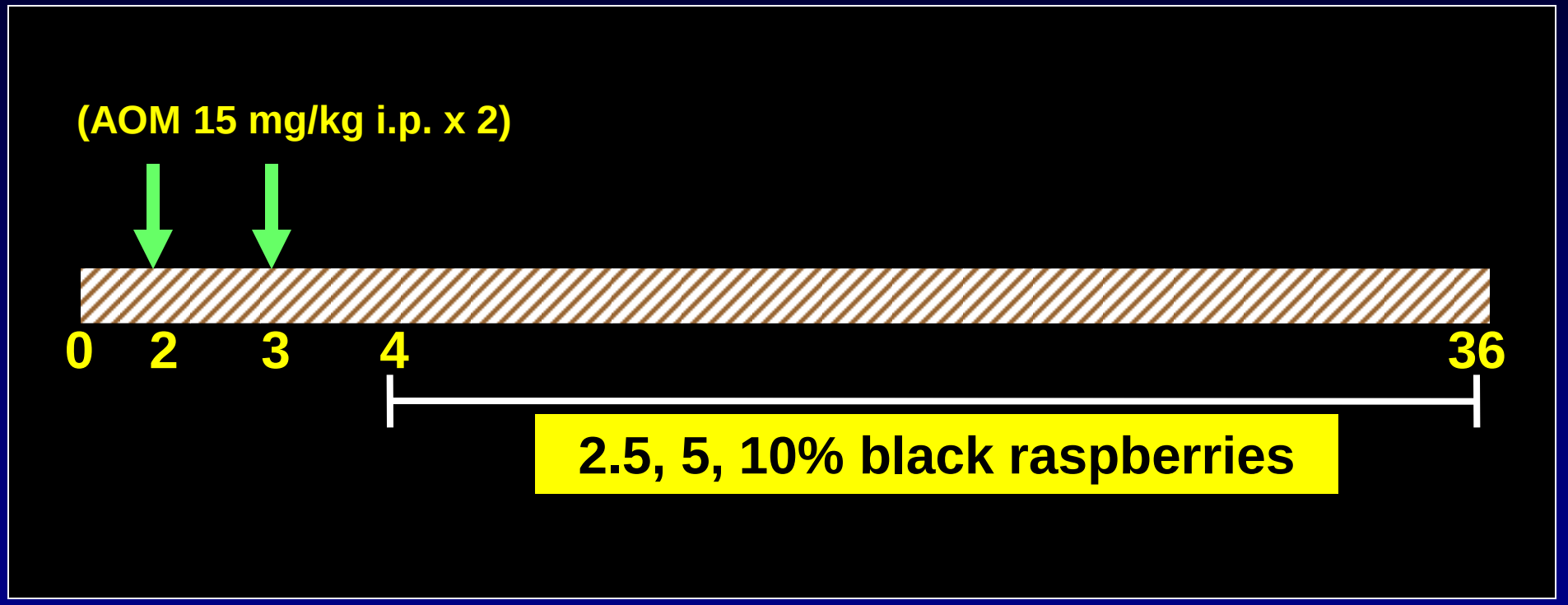


Rat Colon Cancer Model- Azoxymethane (AOM)

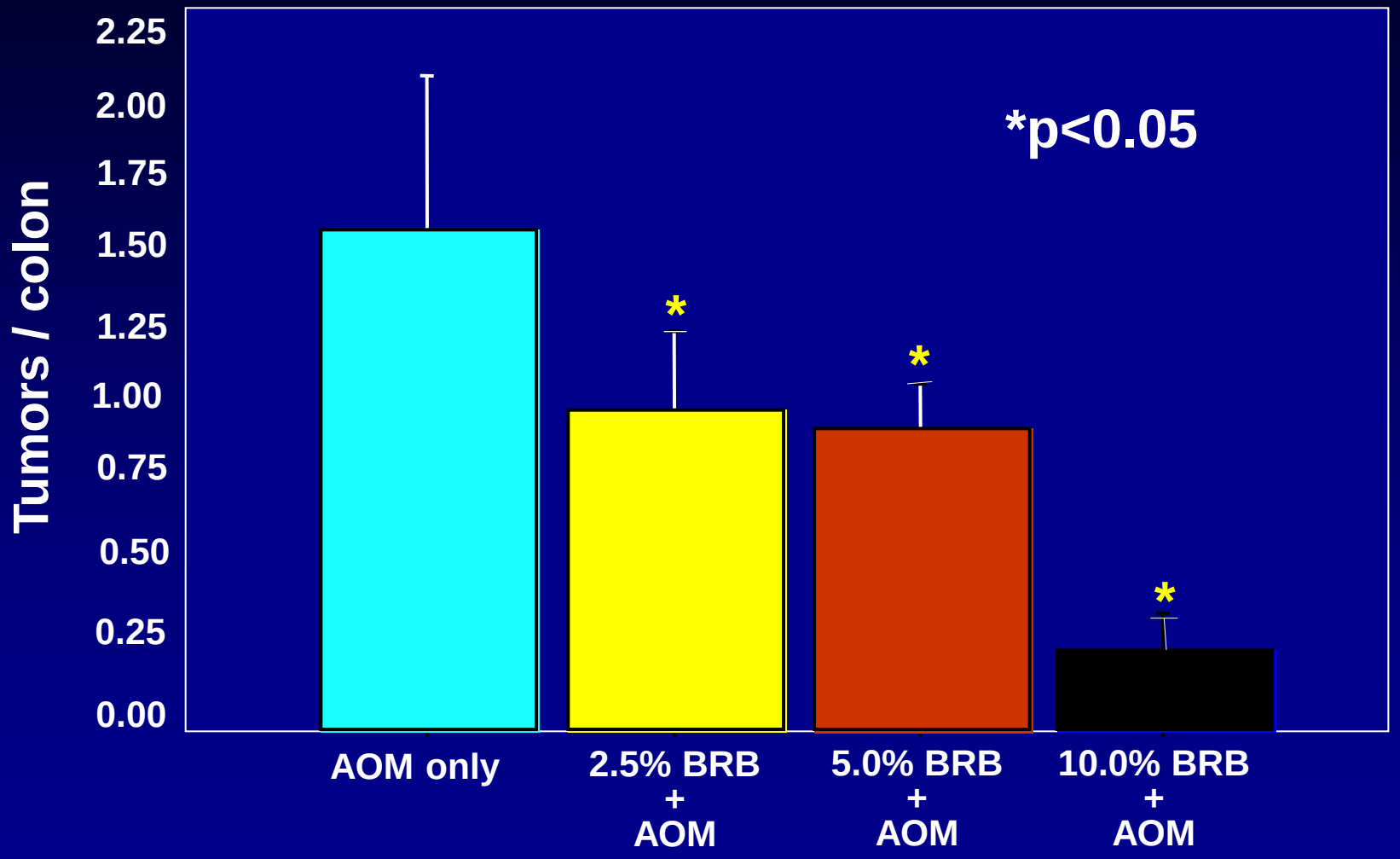




Protocol for AOM-Induced Tumors in Rat Colon



Prevention of Colon Tumors in Rats with BRBs



GK Harris et al., Nutrition and Cancer 40:125-33, 2002

Human Clinical Trials

Phase I Clinical Trial of Freeze-Dried Black Raspberries

- 10 normal volunteers, > 18 years of age
- “Phenol-free” diet
- 45g BRB powder / day, 14 days
- Blood and urine
- Clinical signs of toxicity



Results of Phase I Clinical Trial

- **Berries well tolerated**
- **Uptake of the four anthocyanins and ellagic acid into blood is rapid (maximal at 1-2 hrs).**
- **Overall uptake less than 1% of the administered dose.**

GD Stoner et al., *J Clin Pharmacol.* 45:1153, 2005.

Clinical Trials with Berry Preparations

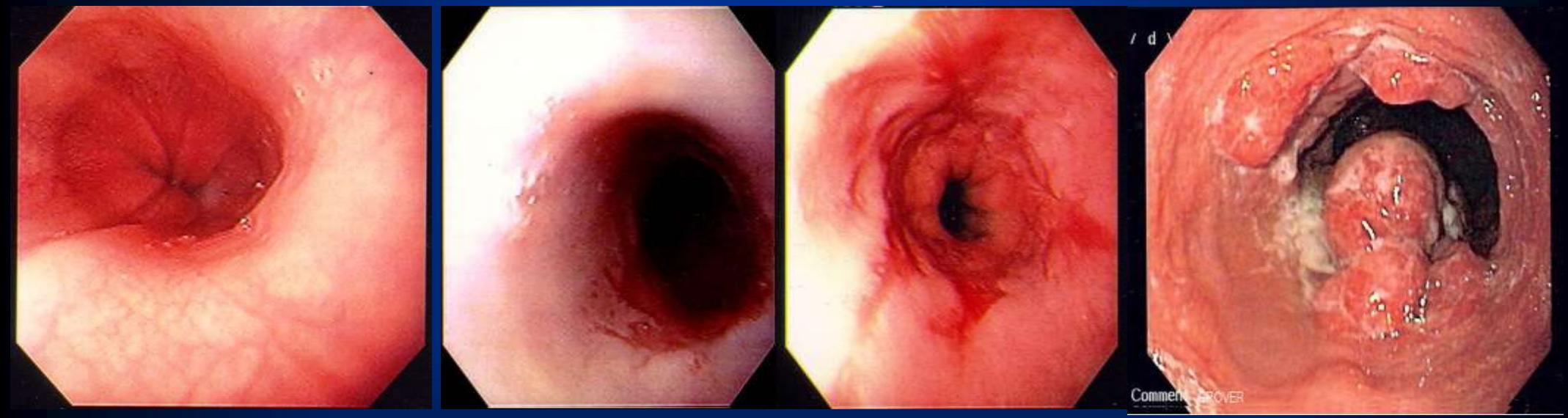
- Barrett's Esophagus – Laura Kresty, Ph.D., John Fromkes, M.D.
- Esophageal Dysplasia – Tong Chen, M.D., Ph.D.
- Colon Cancer – Mark Arnold, M.D., Edward Martin, M.D., Li-Shu Wang, PhD
- Rectal Polyps in FAP patients – Carol Burke, M.D., Li-Shu Wang, PhD
- Oral Dysplasia – Susan Mallery, D.D.S., Ph.D.

Esophageal Adenocarcinoma

Normal

Barrett's Esophagus

Esophageal Adenocarcinoma

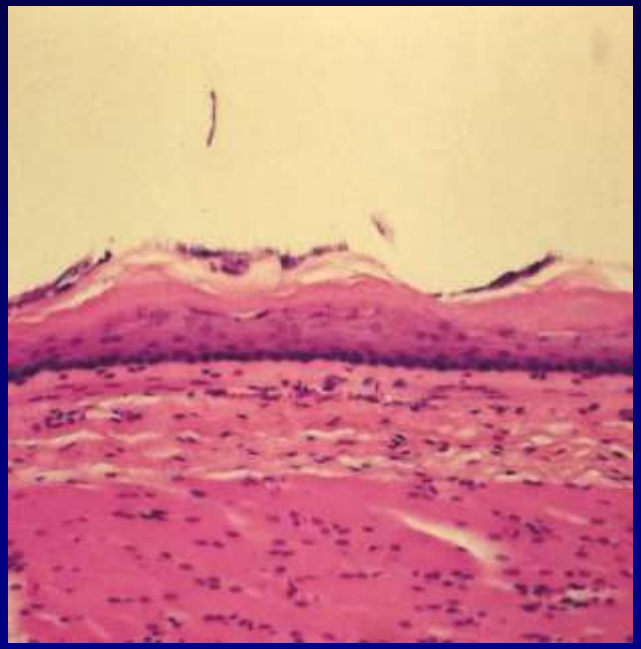


Results

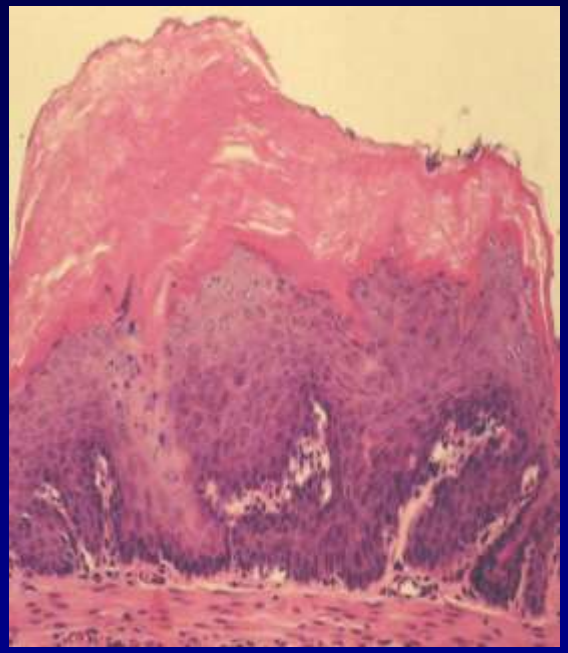
- No reduction in size of Barrett's lesions
- Reduction in cell proliferation ($P = 0.05$)
- Increase in GSTπ ($P<0.05$)
- Reduction in parameters of oxidative stress:
  - urinary isoprostanes
  - urinary 8-0HdG adduct

Esophageal Squamous Cell Carcinoma

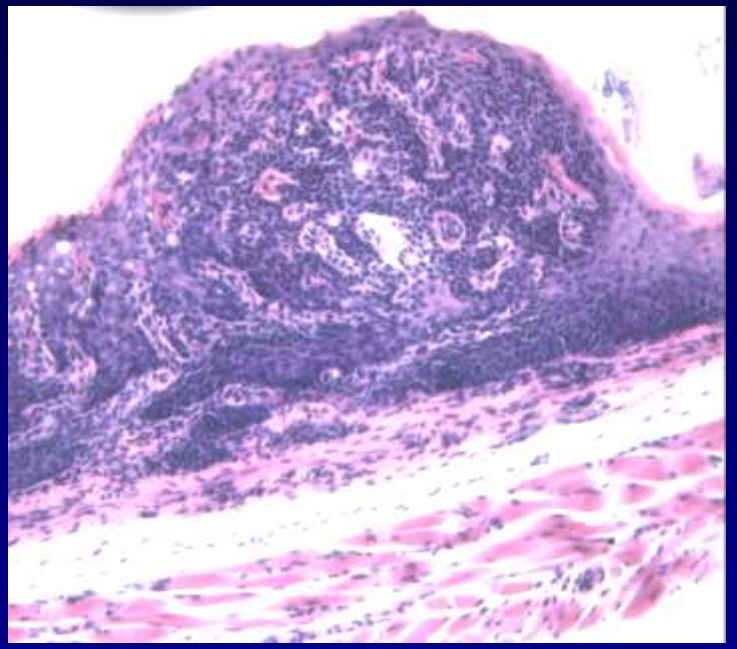
Normal Esophagus



Dysplasia

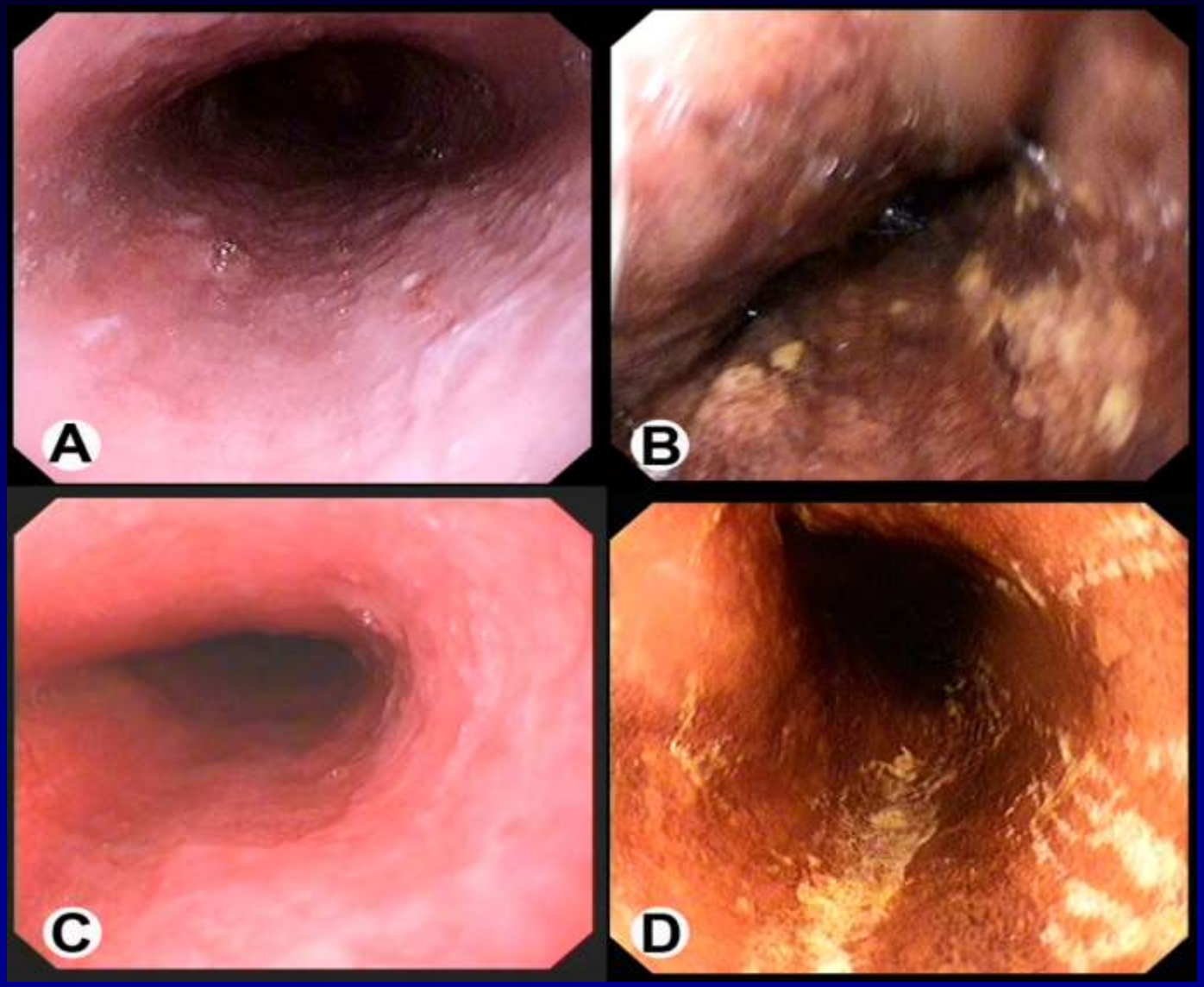


Squamous Cell Cancer



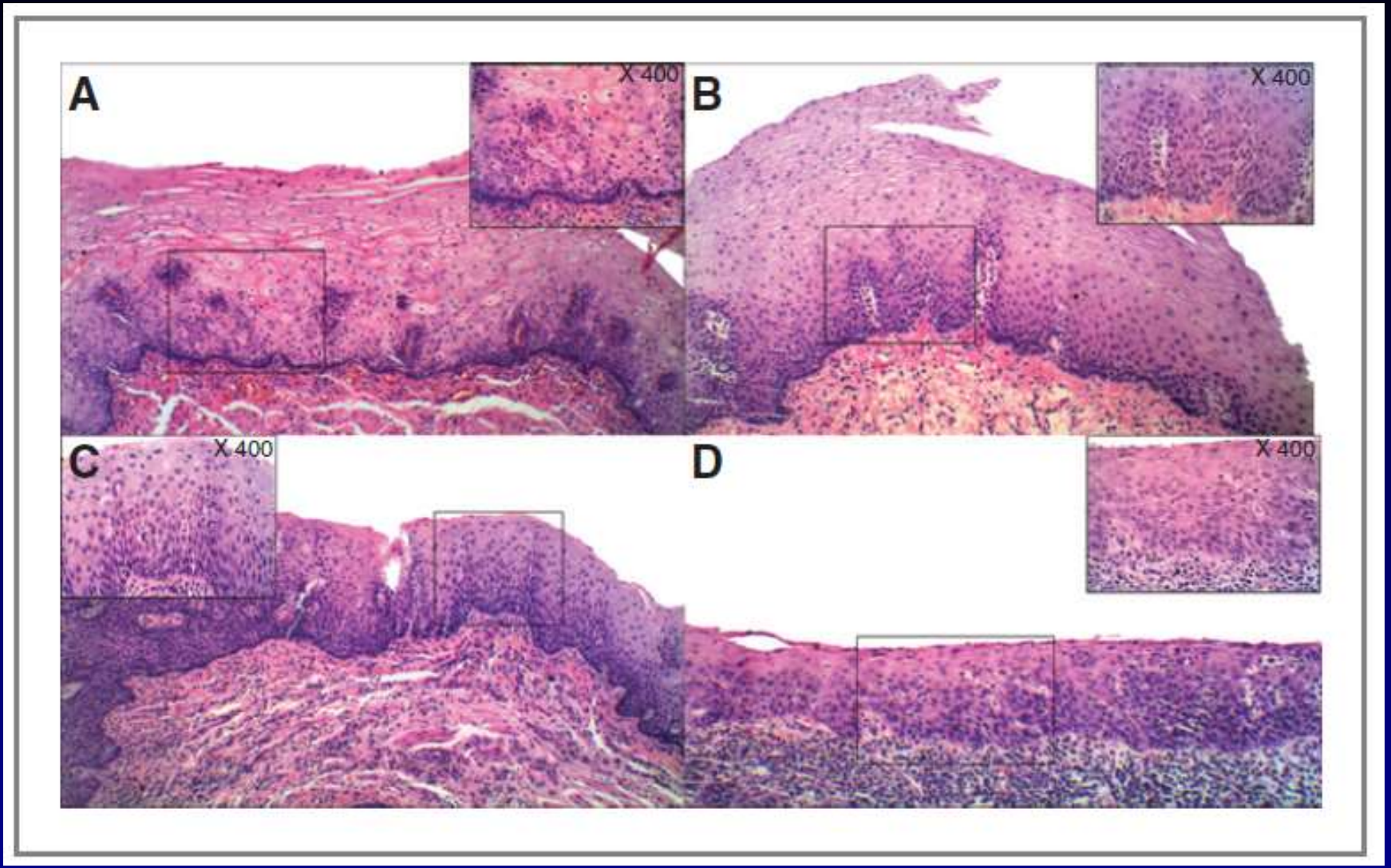

Locations of provinces,
autonomous regions
and municipalities.
Heilongjiang
Jilin
Liaoning
BEIJING
Tianjin
Hebei
Xinjiang
Inner Mongolia
Gansu
Ningxia
Shanxi
Shandong
Qinghai
Henan
Jiangsu
Shaanxi
Shanghai
Tibet
Sichuan
Anhui
Hubei
Zhejiang
Chongqing
Jiangxi
Hunan
Guizhou
Fujian
Yunnan
Guangxi
Taiwan
Guangdong
Hong Kong
Hainan
Islands of
South China Sea

Esophageal Dysplasia



Moderate

Mild



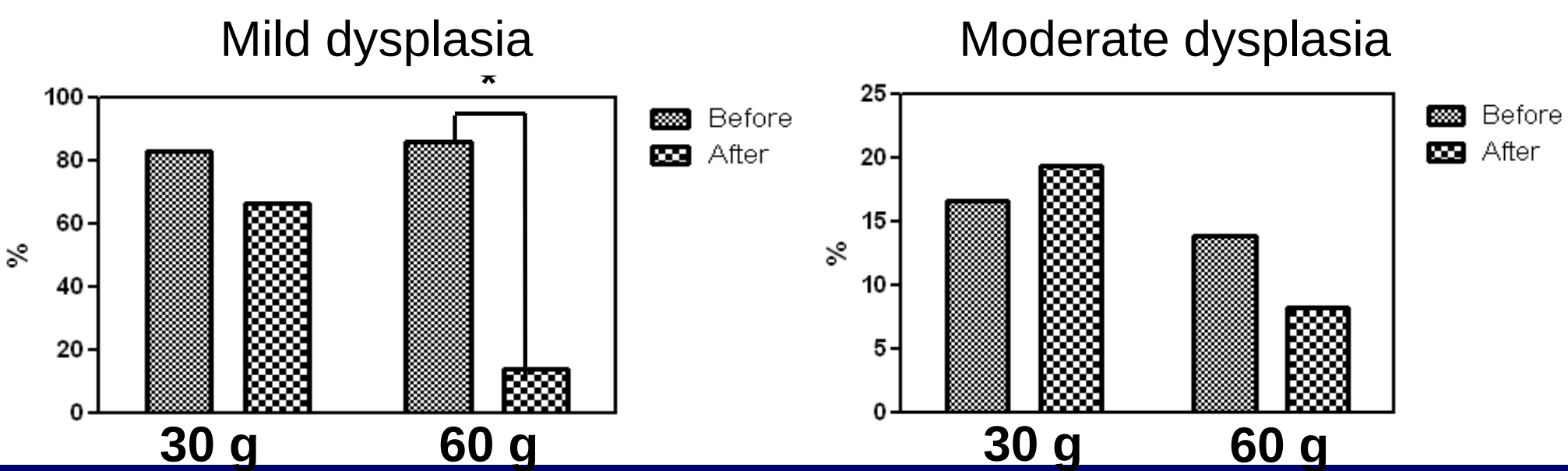
A = normal esophagus; B = hyperplasia; C = mild dysplasia; D = moderate dysplasia

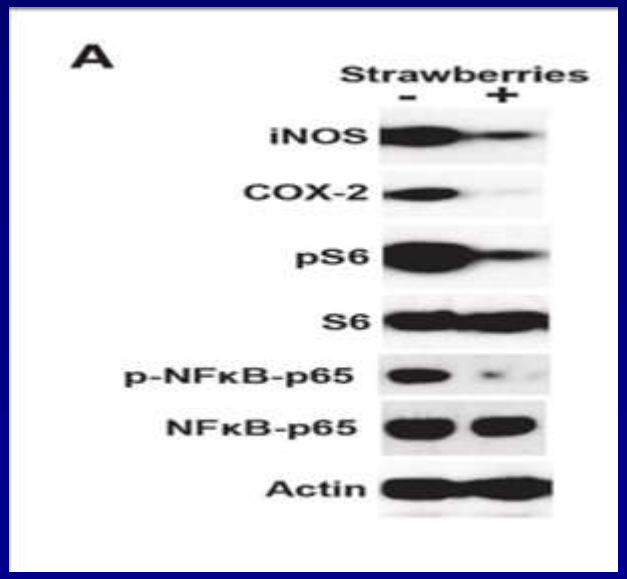
Trial Description

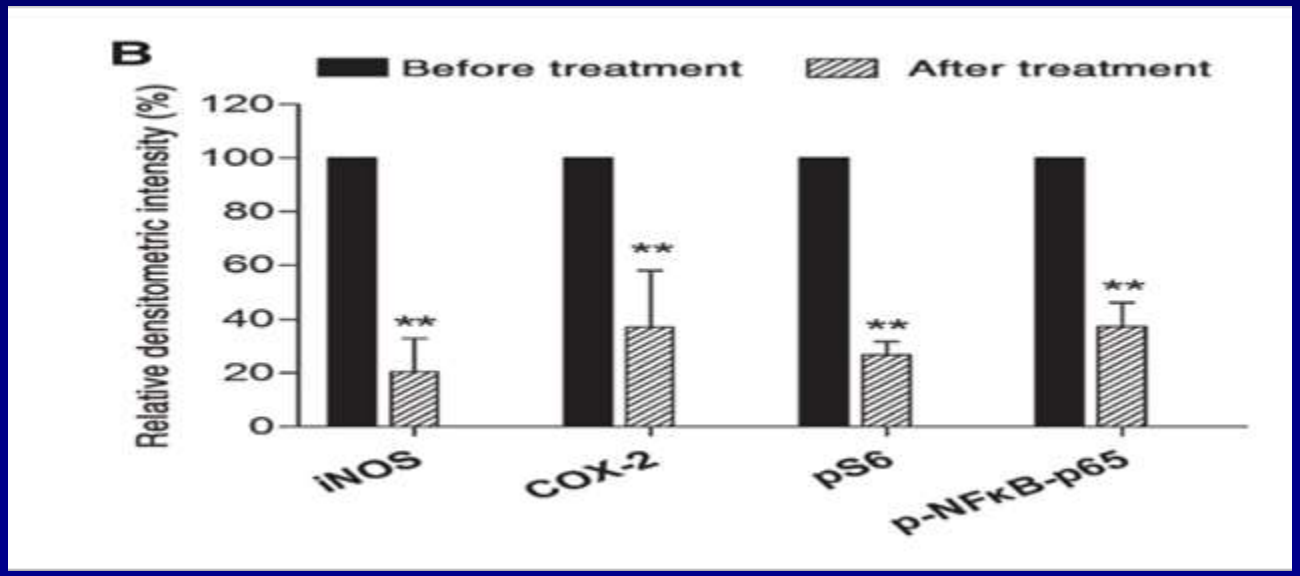
- 75 patients with esophageal dysplasia
- Dose: 30g/day (37) and 60g/day (38) strawberry powder in water
- Treatment period = 6 months
- Lesion biopsies before and after treatment
- Clinical signs of toxicity



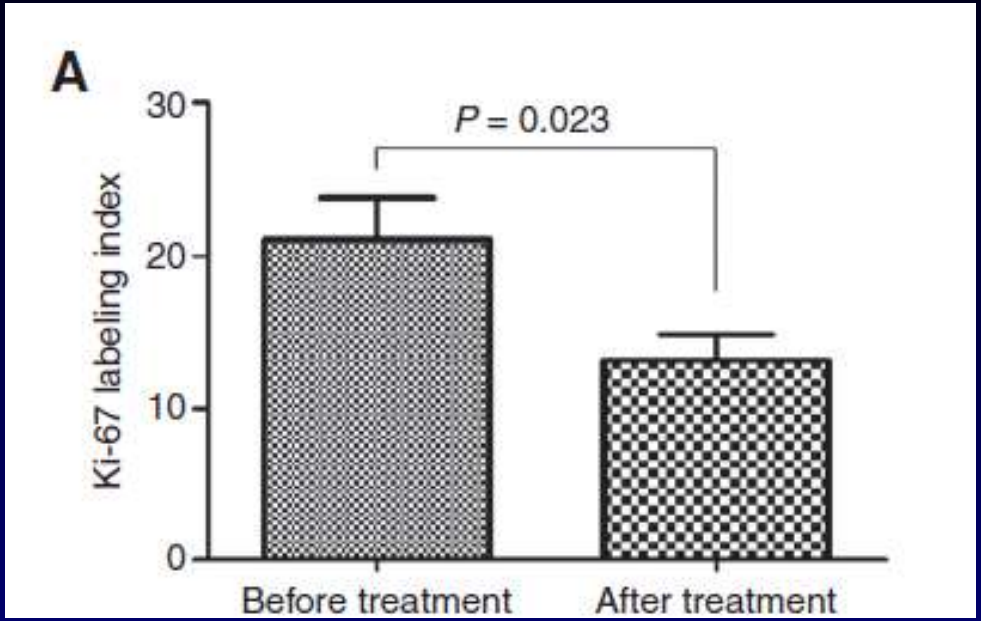
Strawberries reduce esophageal dysplasia in China

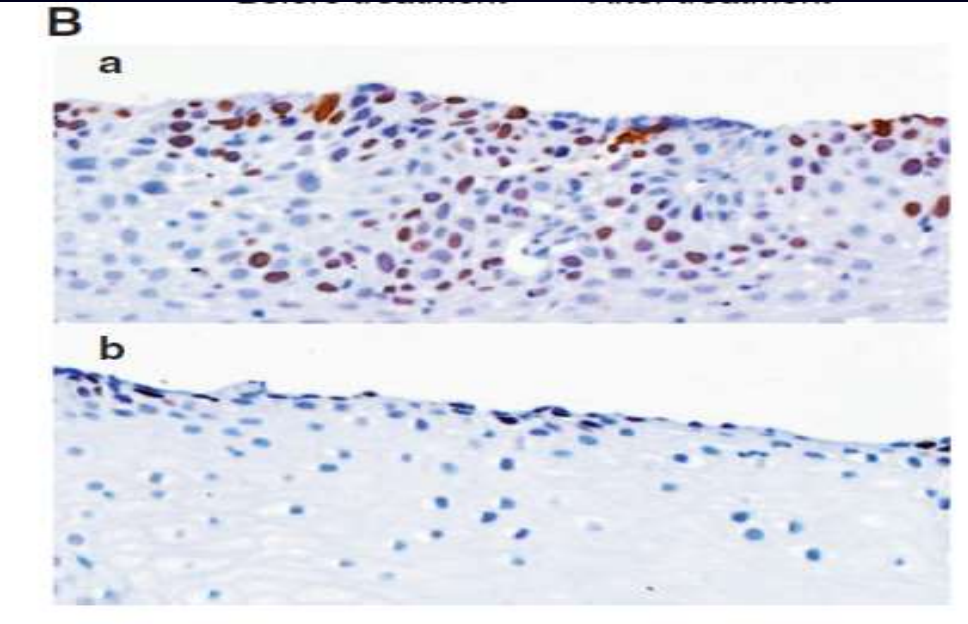






T. Chen et al., *Cancer Prev Res.*5:41 2012





Effect of freeze-dried strawberries on cell proliferation (A). Strawberries significantly reduced Ki-67 expression after 6-month treatment (B, b) when compared with before treatment (B, a; magnification x200)

Tong Chen et al., *Cancer Prev Res* 5:41, 2012

Colon Carcinoma



Colorectal Cancer Study – Pre-Surgical Model

1. Pre-Treatment Colorectal Biopsies
   - •Normal and tumor tissue
2. Blood and Urine Samples

1. Post-Treatment Colorectal Biopsies
   - •Normal and tumor tissue
2. Blood and Urine Samples



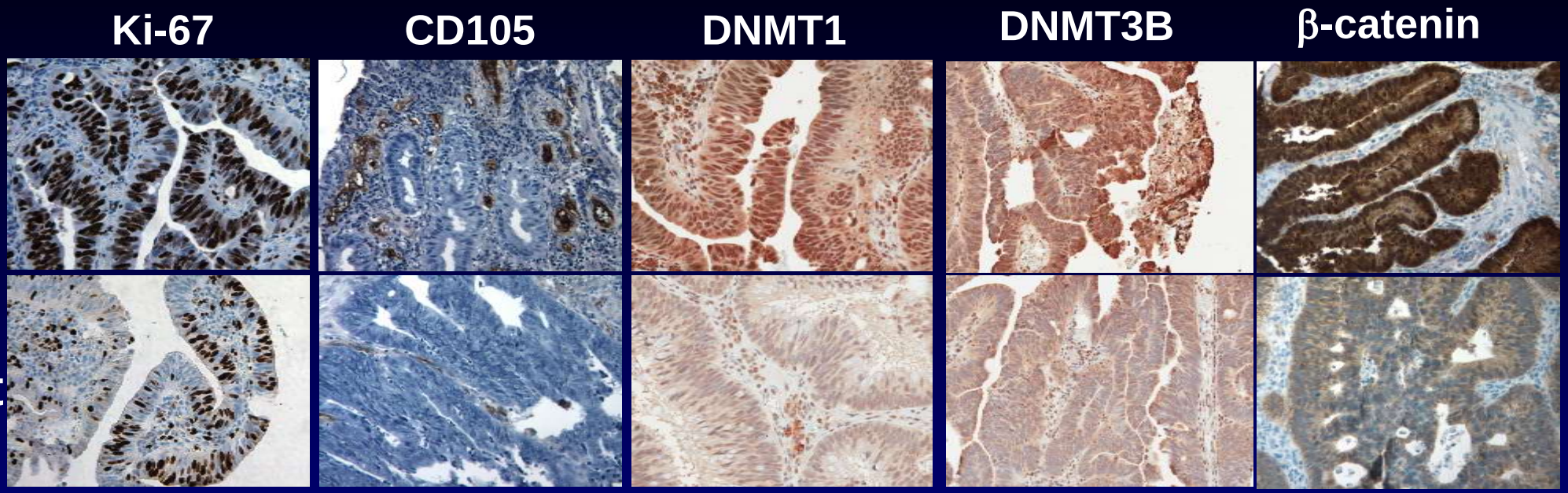


25 Patients - Black Raspberry Slurry (20g/3x/day)



Treatment Time (1-9 weeks)

Berries modulate colorectal cancer biomarkers



Genetic biomarkers
(Protein expression)
↓ β-catenin
↓ E-cadherin
↓ Ki-67/MIB-1
↑ TUNEL
↑ p16
↓ CD105
↓ DNMT1
↓ DNMT3B

Epigenetic biomarkers
(Promoter methylation)
↓ p16
↓ SFRP2
↓ SFRP5
↓ WIF1
↓ PAX6a

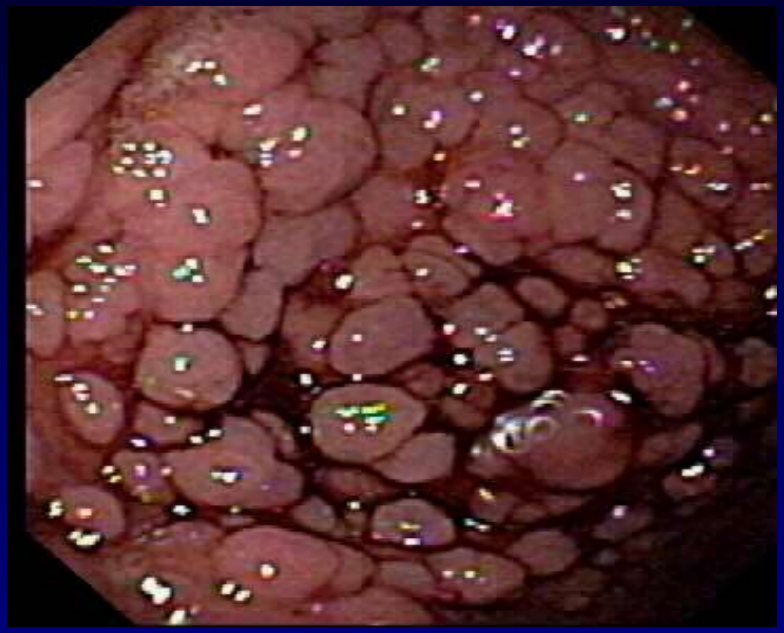


Wang LS et al., Clinical Cancer Res. 2011;17:598.

Familial Adenomatous Polyposis (FAP)



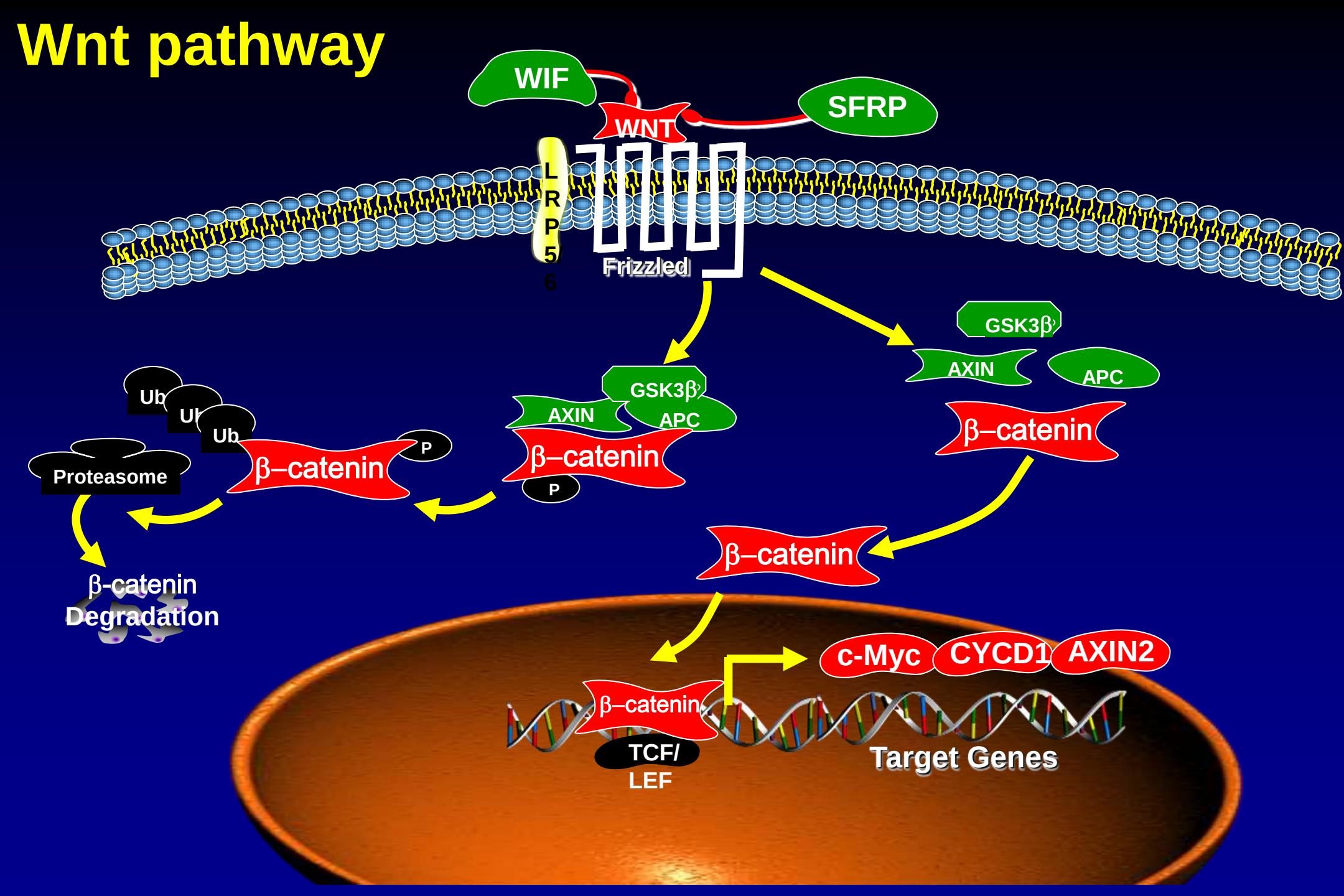
rectum



colon

- Adenomatous polyps in colon with evolution to cancer
- Inherited Apc mutations cause dysregulation of Wnt pathway

Wnt pathway


WIF
SFRP
WNT
LRP5
6
Frizzled
GSK3β
AXIN
APC
β–catenin
P
Ub
Proteasome
β-catenin
Degradation
c-Myc
CYCD1
AXIN2
TCF/
LEF
Target Genes

FAP Study

1. Biopsies
   Normal rectum
   Two polyps
2. Count polyps
3. Blood and urine





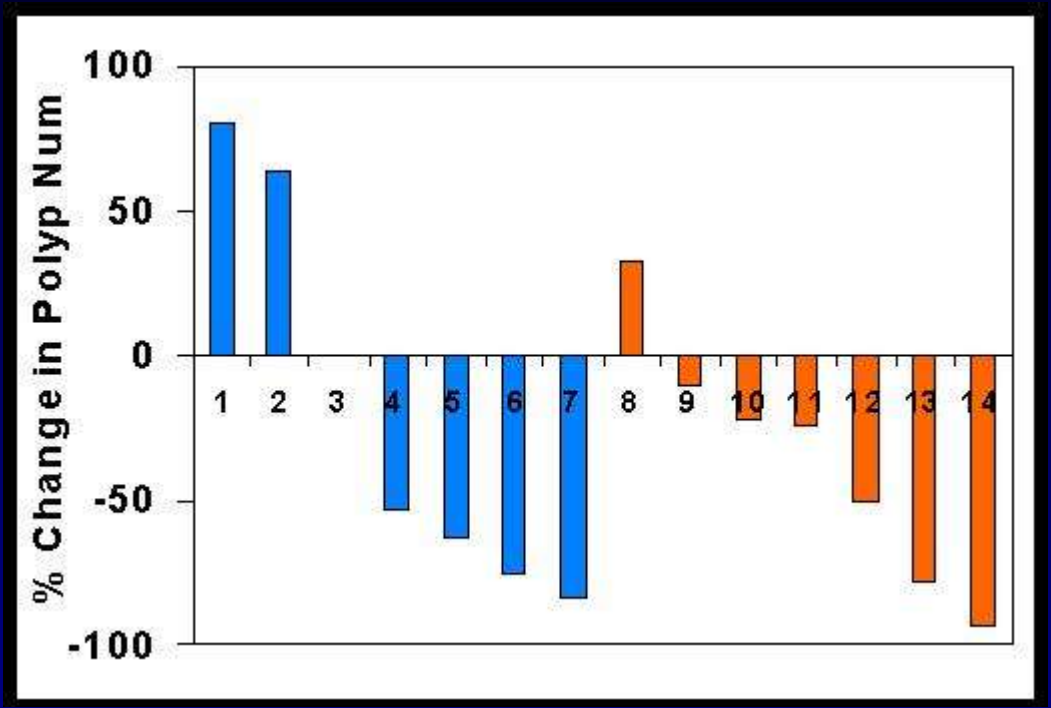
1. Biopsies
   Normal rectum
   Multiple polyps
2. Count polyps
3. Blood and urine

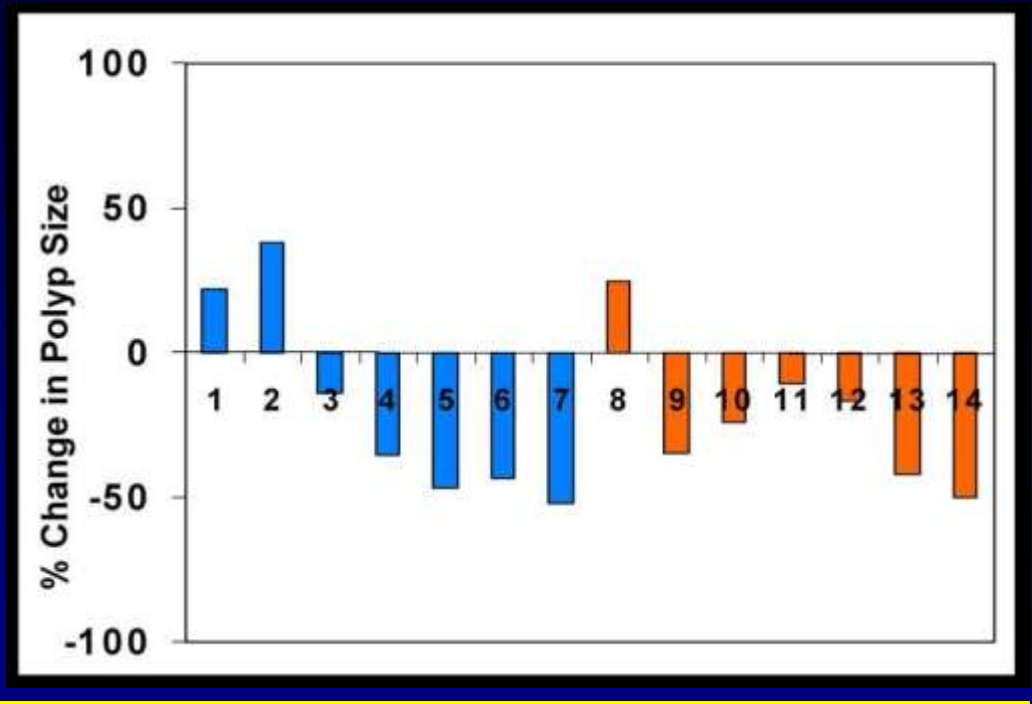
Wk 0 — 36

7 patients - placebo slurry (60 g/day) + BRB suppositories (1.4 g/day)

7 patients - BRB slurry (60 g/day) + BRB suppositories (1.4 g/day)

Effect of Berry Treatment on Polyp Number and Size in FAP Patients

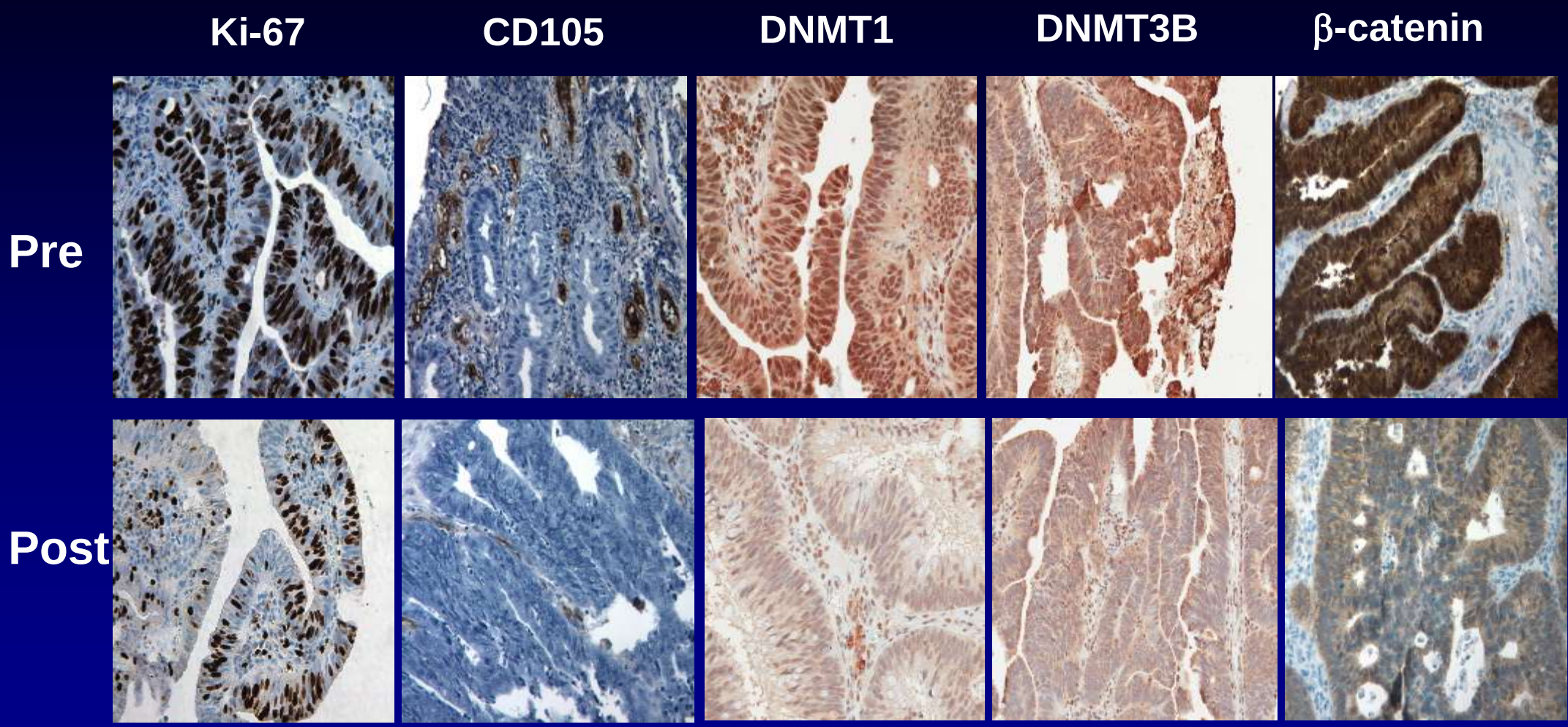




Average reduction in polyp no. (%)	
Oral + Suppos.	19 (Patients 1-7)
Suppos. only	35 (Patients 8-14)
Overall	26

Average reduction in polyp size (%)	
Oral + Suppos.	18 (Patients 1-7)
Suppos. only	21 (Patients 8-14)
Overall	20

Berries modulate colon polyp biomarkers



Wang LS et al., *Clinical Cancer Res.* 2011;17:598.



Trial Description

- 10% BRB gel applied to dysplastic oral lesions of 22 patients 4X/day for 3 months (total 2 g/d).
- Placebo gel applied to dysplastic lesions of 18 patients 4X/day for 3 months.



Results of Oral Dysplasia Trial

Topical application of 10% black raspberry gel, 4X/day for 3 months resulted in:

- **Reduction in lesion size in 60% of cases**
- **Significant reduction in histologic grade in 70% of cases**
- **Return of heterozygosity at tumor suppressor gene loci in ~67% of cases.**

B. Shumway, et al., *Clin Cancer Res*., 14:2412, 2008; S. Mallery, et al., *Cancer Res* 68:4945, 2008; S.Mallery, et al., *Clin Cancer Res* 20:1910, 2014

Conclusions

In humans, berries:

- **Are well tolerated when administered in different formulations.**
- **Reduce cell proliferation in Barrett's esophagus and parameters of oxidative stress.**
- **Regress mildly dysplastic squamous lesions in human esophagus**
- **Reduce cell proliferation, angiogenesis, DNA methylation and rectal polyp number in FAP patients**
- **Modulate biomarkers of lesional development in the oral cavity.**

Collaborators-Berry Studies

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