Mechanisms of colorectal and lung cancer prevention by vegetables: a genomics approach

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Outline

- Background: vegetables and cancer prevention;
- Overview of experimental studies investigating gene and/or protein expression changes in the colon and lung;
- Modulated pathways;
- Example: Human dietary vegetable intervention study in the colon;
- Conclusions & take home messages.

Van Breda *et al.* Mechanisms of colorectal and lung cancer prevention by vegetables: A genomic approach. Journal of Nutritional Biochemistry, 2008;19:139-157

Evidence vegetables and cancer risk reduction: WCRF 1997

	Convincing	**Probable**	**Possible**
Vegetables in general 	Stomach **Colon and rectum** Esophagus **Lung** Oral cavity and Pharynx	Larynx Pancreas Breast Bladder	Ovary Endometrium Cervix Liver Prostate Kidney
Allium vegetables  	Stomach	**Colon** **Rectum**	
Tomatoes 	Stomach Esophagus **Lung**	**Colon** Oral cavity and pharynx **Rectum**	
Green (leafy)  	Stomach **Colon** Esophagus **Lung** Oral cavity and pharynx	Breast Bladder	

Evidence vegetables and cancer risk reduction: WCRF 2007

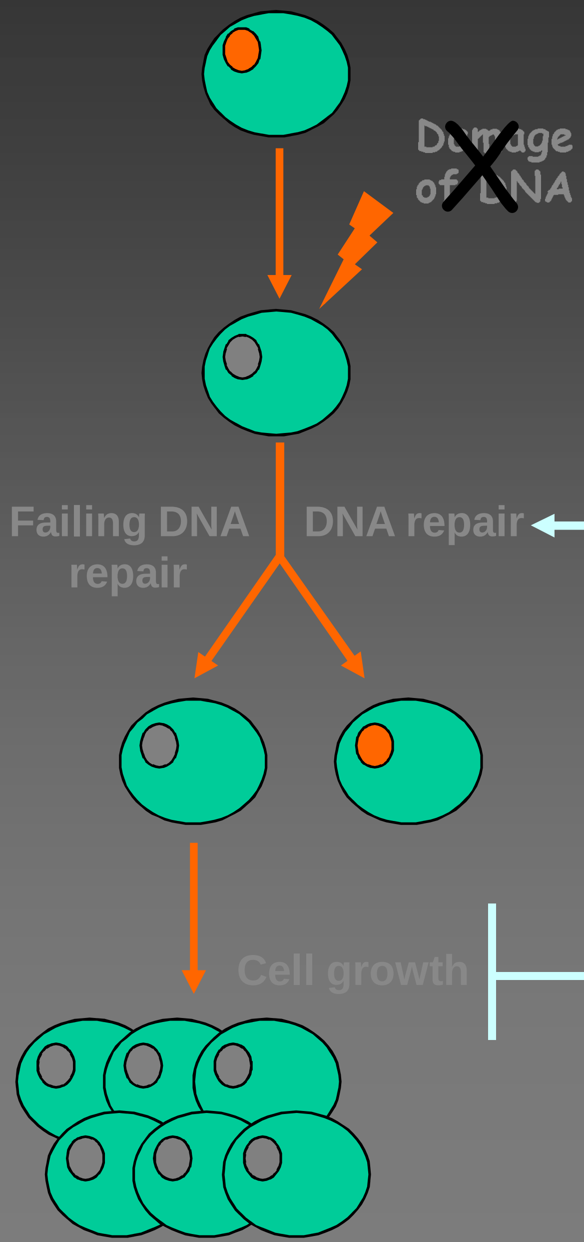
Decrease risk		
Evidence	**Exposure**	**Cancer site**
Convincing		
Probable	Non-starchy vegetables Allium vegetables Garlic Foods containing folate Foods containing carotenoids Foods containing beta-carotene Foods containing lycopene Foods containing vitamin C Foods containing selenium	Mouth, pharynx, larynx, oesophagus, stomach Stomach **Colorectum** Pancreas Mouth, pharynx, larynx, **lung** Oesophagus Prostate Oesophagus Prostate
Limited-Suggestive	Non-starchy vegetables Carrots Pulses Foods containing folate Foods containing pyridoxine Foods containing vitamin E Foods containing selenium Foods containing quercetin	Nasopharynx, **lung, colorectum**, ovary, Cervix Stomach, prostate Oesophagus, **colorectum** Oesophagus Oesophagus, prostate **Lung**, stomach, **colorectum** **Lung**

Epidemiology: evidence weakened since 1997

- Michels KB et al. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. J Natl Cancer Inst 2000;92:1740-52.
- Voorrips LE et al. Vegetable and fruit consumption and risks of colon and rectal cancer in a prospective cohort study: The Netherlands Cohort Study on Diet and Cancer. Am J Epidemiol 2000;152:1081-92.
- Feskanich D et al. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. J Natl Cancer Inst 2000;92:1812-23.
- Hung HC, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, et al.Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst 2004;96:1577-84. **Nurses Health Study**

→ In addition to epidemiological studies, experimental research using sensitive biomarkers is needed to investigate the causal relationship between vegetables and cancer.

Cancer (Phytochemicals from) vegetables



Blocking mechanisms: inhibition of DNA damage and mutation

- Prevention of carcinogen uptake
- Inhibition of enzymatic carcinogen formation/activation: modulation of biotransformation enzymes
- Blocking, quenching or scavenging of reactive metabolites
- Induction of DNA repair and -synthesis

Suppressing mechanisms: suppression of promotion, progression, invasion and metastasis

- Inhibition of cell proliferation
- Induction of apoptosis
- Induction of cell differentiation
- Protection of intercellular communication
- Modulation of immune system
- Inhibition of tumor induced neo-vascularization
- Inhibition of metastasis: activation of antimetastasis genes

General hypothesis

An important contribution of the anticarcinogenic effects of vegetables in the colorectum and lung is through modulating the expression of genes involved in biological and genetic pathways that are relevant for carcinogenesis

Experimental studies:
vegetables and gene expression changes in the **COLON**

***In vitro*:**

- Colorectal cellines: HT-29, Caco-2, LS-174,…,…;
- Single vegetable compounds: sulphoraphane, quercetin, indoles, beta-carotene;
- Number of genes investigated at once is limited (pre-defined genes); however, increase in number of studies using microarray technology.

***In vivo*: Animal studies**

- Mouse is most often used;
- Single vegetable compounds: organosulpur compounds, quercetin; and whole vegetables: broccoli, cauliflower, carrots, peas, onions;
- Number of studies is limited, most of them investigate a limited number of genes (pre-defined genes).

***In vivo*: Human studies**

- Number of studies is very limited (3);
- Whole vegetables: Brussels sprouts, broccoli, vegetables mixture (cauliflower,carrots, peas and onions);
- only one study used microarrays for gene expression analyses.

Experimental studies:
vegetables and gene expression changes in the **LUNG**

***In vitro*:**

- Lung cellines: A549 human adenocarcinoma, NCI-H209 carcinoma, C10, E9, and 82-132 murine epithelial cells;
- Single vegetable compounds: flavone, quercetin, beta-carotene;
- Number of genes investigated at once is limited (pre-defined genes).

***In vivo*: Animal studies**

- Rat is most often used;
- Single vegetable compounds: organosulphur compounds, glucosinolate; indole-3 carbinol, isothiocyanates, and whole vegetables: broccoli, cauliflower, carrots, peas, onions;
- Number of studies is limited, most of them investigate a limited number of genes (pre-defined genes).

***In vivo*: Human studies**

- No human studies have been reported.

Experimental studies investigating the effect of vegetable (components) on gene and/or protein expression

	In vitro	*In vivo* Animal	*In vivo* Human
Colon	**12 (3)**	**7 (2)**	**3 (1)**
Pathways	Cell cycle, apoptosis, cell proliferation, biotransformation of xenobiotics, intracellular defense	Apoptosis, intracellular defense, cell proliferation	Intracellular defense, cell cycle, cell proliferation, biotransformation of xenobiotics
Lung	**3**	**4 (2)**	**-**
Pathways	Cell proliferation, intracellular communication, apoptosis	Biotransformation of xenobiotics, cell proliferation, apoptosis, immune response	-

* Numbers between brackets represent “omic” appraoches

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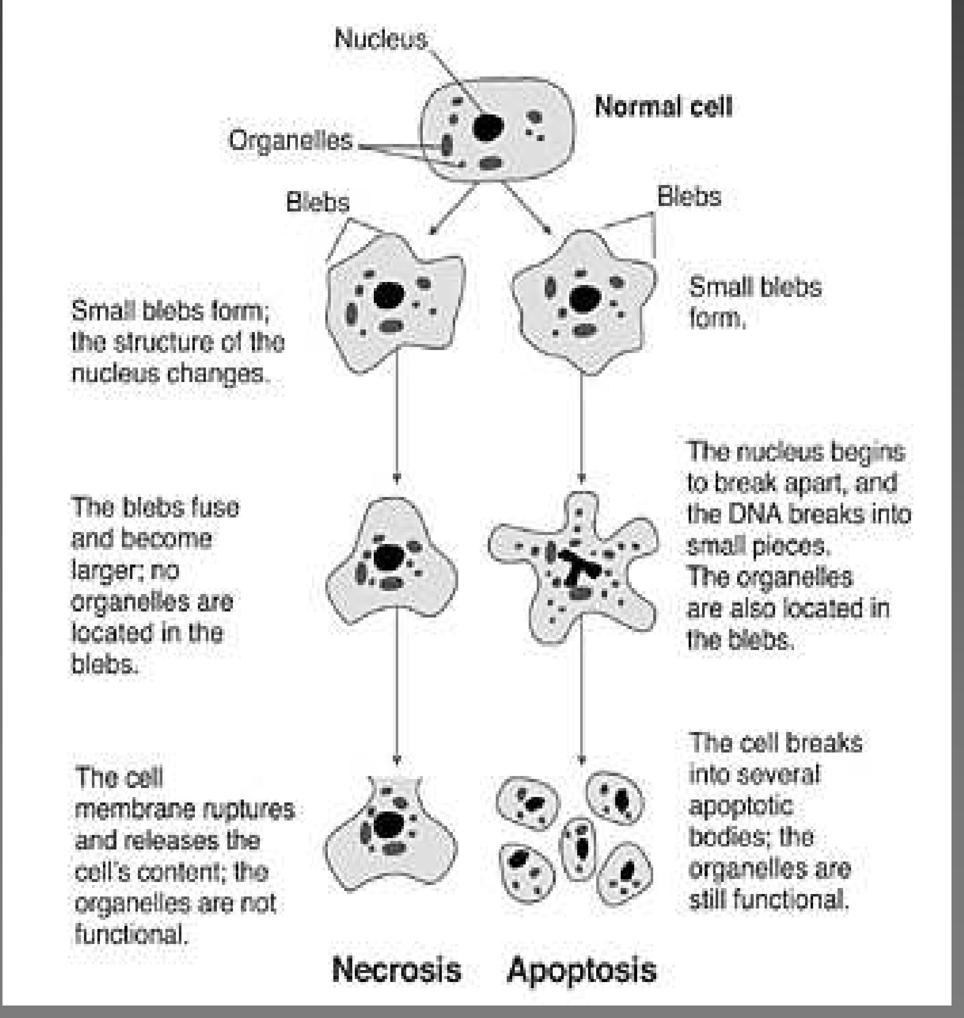
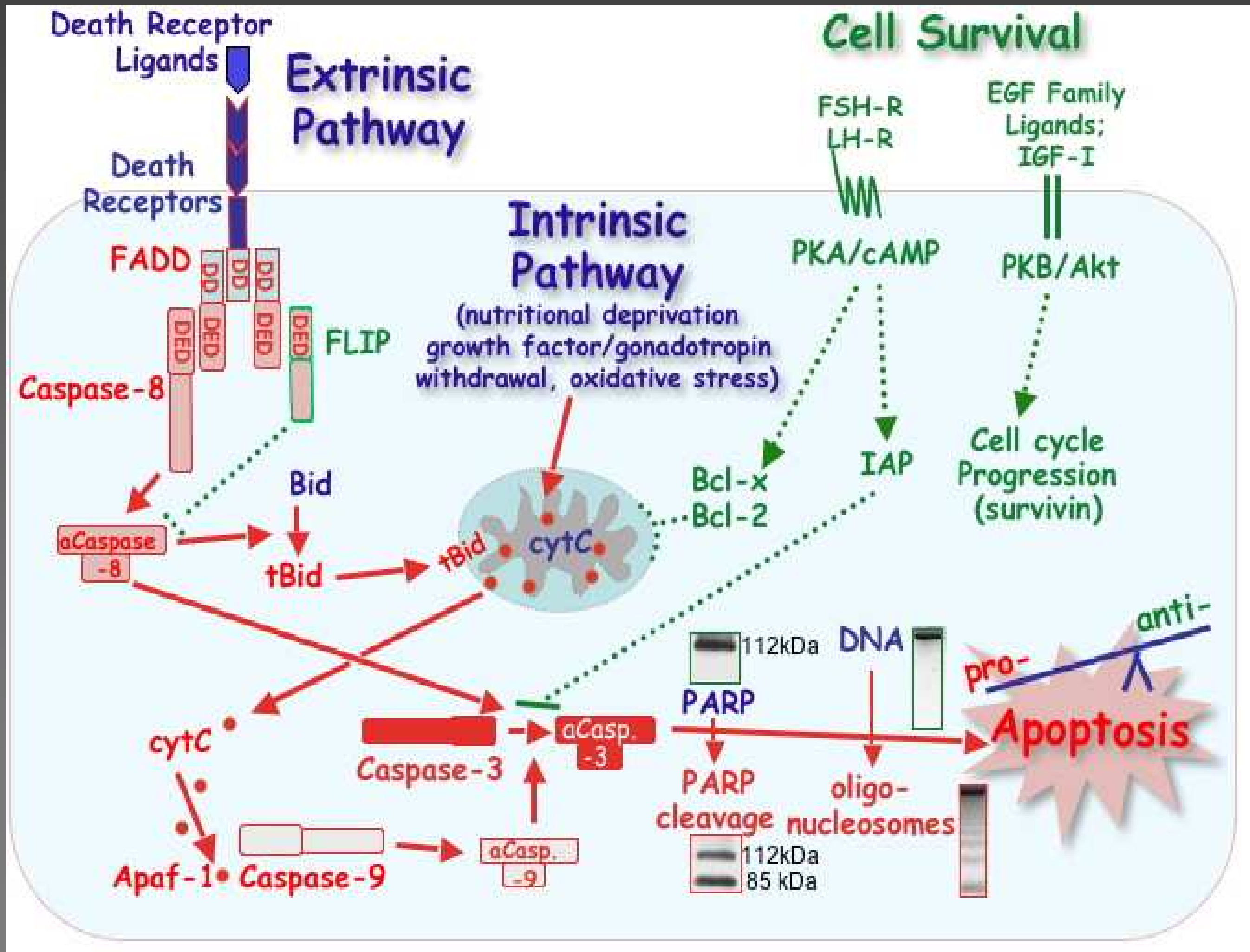
Induction of apoptosis

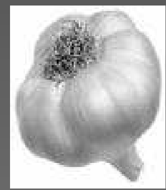


- programmed cell death
- orderly process that eliminates cells which become malfunctioning;
- plays an important role in cell homeostasis: cell number is kept constant through a balance between cell division and cell death.

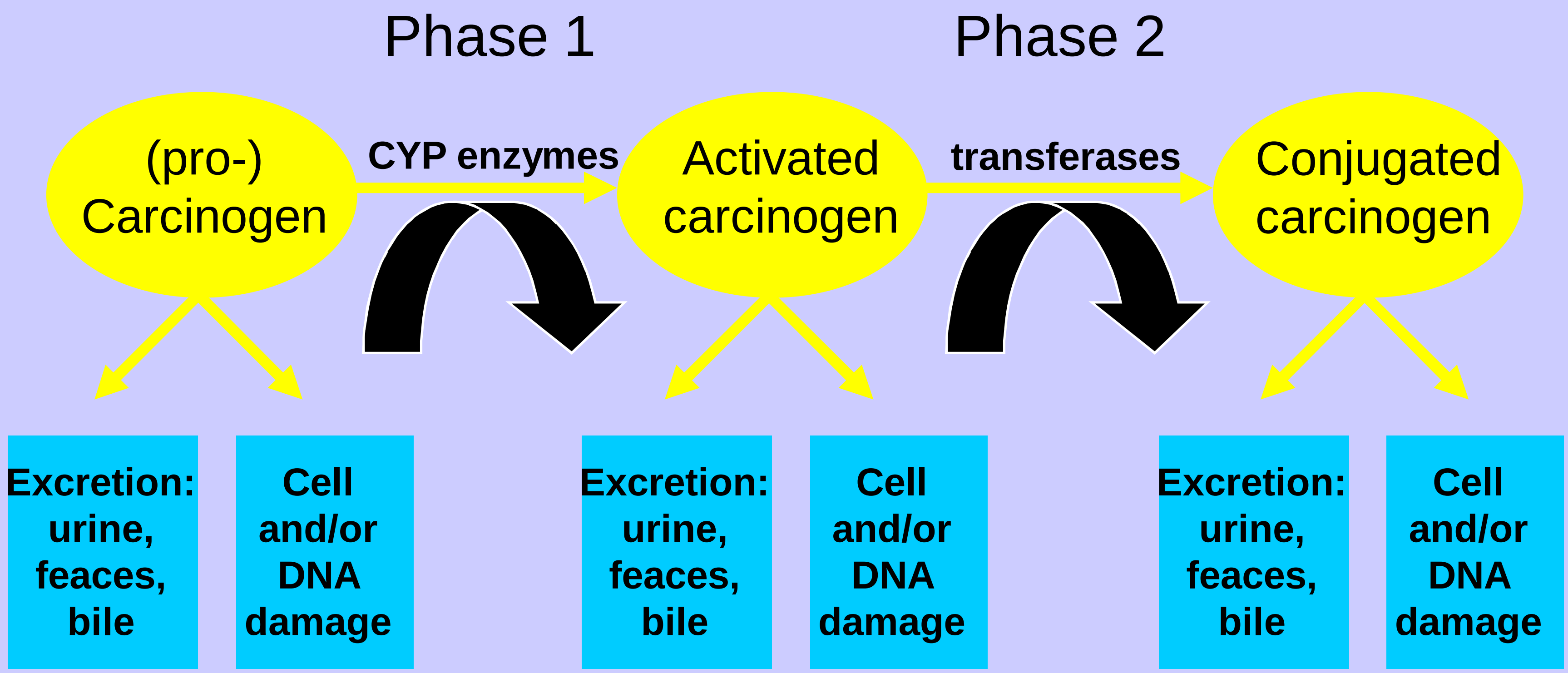
Induction of apoptosis



Induction of apoptosis: anticancer: elimination of genetically damaged cells before they can undergo clonal expansion

Compound	Action	Vegetable source
Flavonoids	Induction of BAK, reduction of BCL-X_L (colon, lung), activation of caspases (colon, lung), inhibition of BNIP1 (colon)	 
Isothiocyanates	Induction of GADD, BAK, and caspases (colon), induction of BNIP1 (lung)	 
Indoles	Induction of caspases (colon)	 

Biotransformation: activation and deactivation of (pro-) carcinogens takes place by several enzyme systems:



primairy goal: to make substances more polar (water soluble) to facilitate excretion → negative side effect: activation of carcinogens

Phase 1 enzymes: oxidation, reduction and hydrolysis

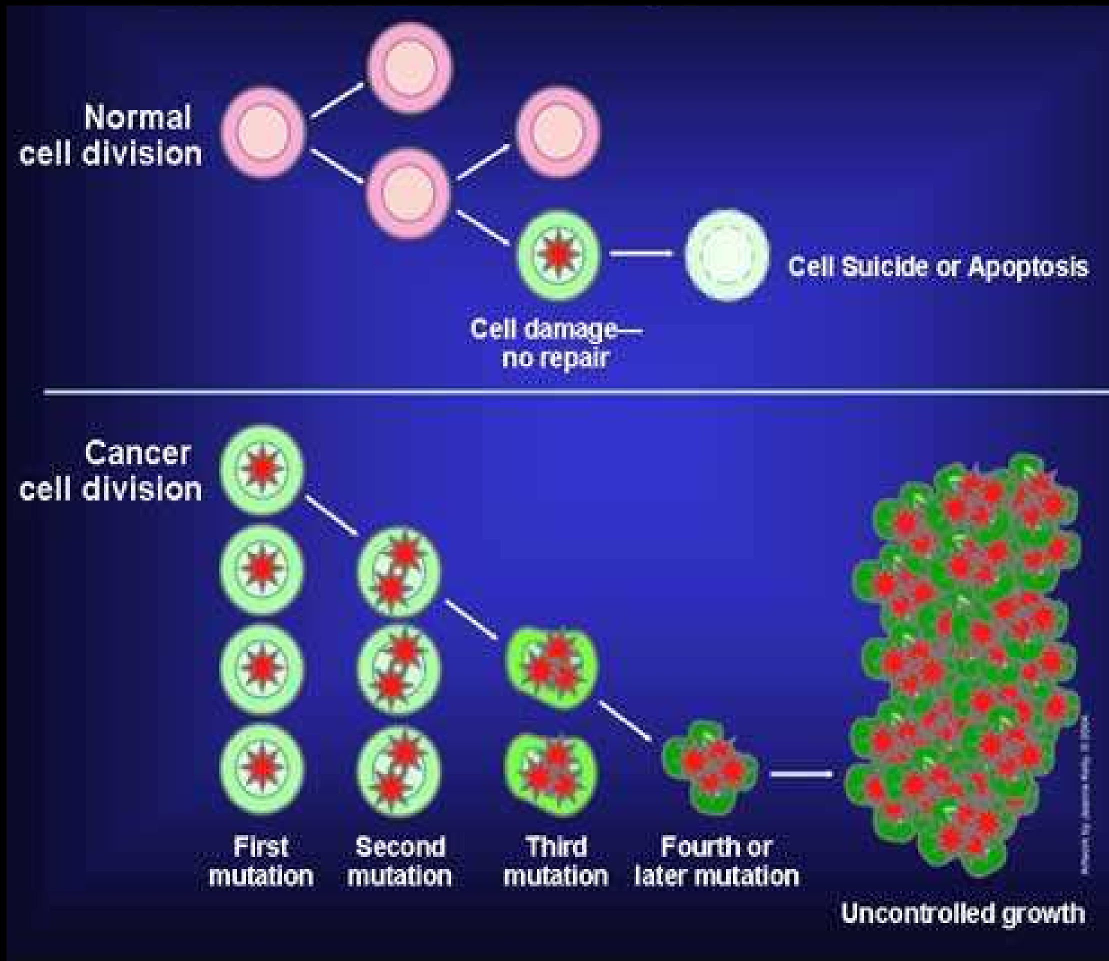
→cytochrome P450 enzymes in (liver), lung and colon

Vegetable	Compound	Effect on mRNA/enzyme
	glucosinolates	Induction of CYP1A1, CYP1A2, CYP3A1, CYP3A2, CYP2B1, CYP2B2, CYP2C11 (lung)
	Indoles	Induction of CYP1A1, reduction of CYPIIB (colon)
	Flavonoids Organosulfur compounds	Induction of CYP4FII, CYP3A5 (colon)

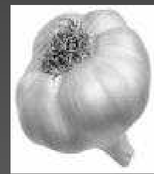
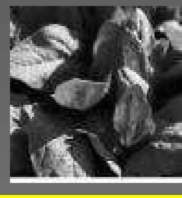
Phase 2 enzymes: conjugation reactions with the phase 1 metabolites $\rightarrow$ glutathione S transferases, N-acetyltransferases, UDP-glucoronyltransferases, sulfotransferases

Vegetable	Compound	Effect on mRNA/enzyme
	Isothiocyanates	Induction of AKR1C1 (colon)
	Indoles	Induction of NQO1, AKR1C1 (colon), induction of GSTμ and –π (colon)
	Organosulfur compounds	Induction of UDP-glucoronyl transferase, microsomal epoxide hydrolase, quinone reductase (colon)

Inhibition of cell proliferation



Inhibition of cell proliferation

Compound	Effect on mRNA/enzyme	Vegetable source
Flavonoids	Induction of p21 (colon, lung), inhibition of cycle 2-cyclin B (colon), induction of cyclin B (lung)	 
carotenoids	Inhibition of cyclin A (colon), downregulation of GLUL (lung)	 
Indolen	Inhibition of cyclin B1, induction of p21 (colon)	
sulforaphane	Inhibition of MYCBP, Cyclin D1, -A, -E; upregulation of p21 (colon)	 

Experimental studies: vegetables and gene expression changes in the colon and lung

- Human dietary intervention study (colon):

  high and low level of a mix of vegetables

  (v*an Breda et al. Carcinogenesis 2004;25(11):2207-16)*

- Mouse nutrition study (colon and lung):

a) Dose-response of a vegetable mix

b) Specific vegetables

(van Breda et al. The Journal of Nutrition 2005;135:1879-88)

(van Breda et al. The Journal of Nutrition 2005;135:2546-2552)

- DNA microarrays:

a) Commercial arrays

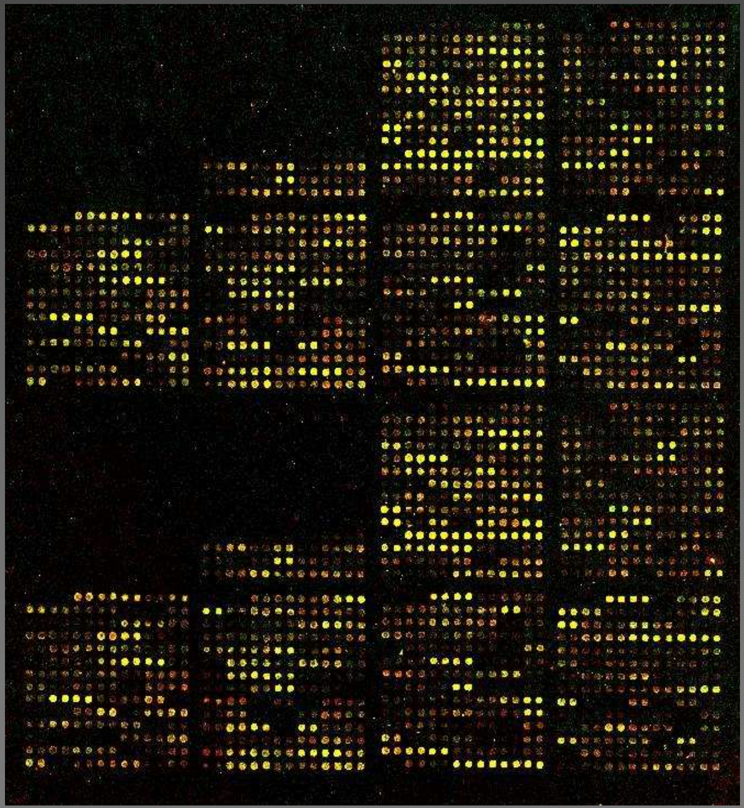
b) Home made arrays

Microarrays used

1. Human study:

PHASE-1 microarray Human 600

- Commercial arrays
- 600 human genes which address toxicologically relevant gene pathways



2. Mouse studies:

Mouse variants of PHASE-1 microarray Human 600

- Home made arrays
- 600 mouse genes

Human dietary intervention study

- Modulation of gene expression by vegetables in colorectal mucosa of sporadic adenoma patients and healthy controls
- Identification of genes that are modulated *in vivo* in colorectal epithelium by vegetables
- Investigate whether the effect of vegetables is different in colon adenoma patients compared to controls

Approach

Study population

Patients (N=20)

10 patients 300 g/day

10 patients 75 g/day

Controls (N=8)

4 controls 300 g/day

4 controls 75 g/day

Studydesign

1 14 15

PRE-INTERVENTION
Biopsies: gene expression

14 DAYS INTERVENTION

POST-INTERVENTION
Biopsies: gene expression

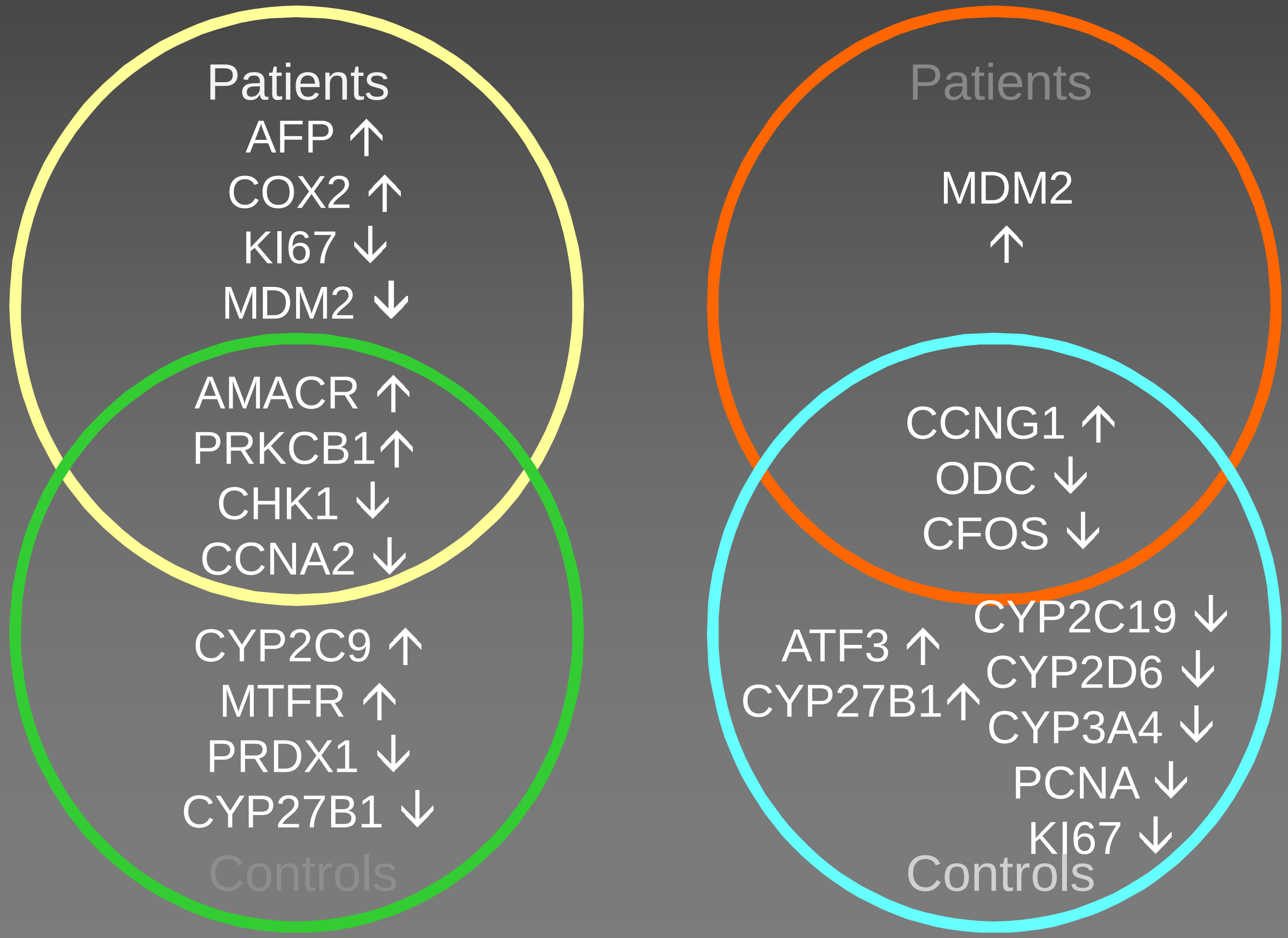
Differentially expressed genes

Confidence analysis (up and down regulation level 0.25 and -0.25 respectively): number of genes				
	75 g/day		**300 g/day**	
	Up	**Down**	**Up**	**Down**
Patients	13	10	7	2
Controls	15 (5)	12 (6)	10 (4)	8 (2)

- In total 58 different genes
- 17 similar response in both patients and controls
- 20 genes are known to be related to (colon)carcinogenesis

Differentially expressed genes related to CRC

75 g/day **300 g/day**



Theoretical correlations gene expression and CRC risk

Gene	Decreased vegetable intake		Increased vegetable intake		Involved pathway
	Effect on expression	Theoretical effect on CRC	Effect on expression	Theoretical effect on CRC	
AMACR	+	+			Metabolism
ODC1			—	—	
PKCB1	+	+			Cell cycle/growth
CCNA2	—	—			
CCNG1			+	—	
MDM2	—	?	+	?	
CHK1	—	+			
C-FOS			—	—	
COX-2	+	+			
CYP2C9	+	+			Oxidoreductase activity
CYP2C19			—	?	
CYP2D6			—	?	
CYP3A4			—	—	
CYP27B1	—	+	+	—	

→ Almost all expression changes are correctly linked to cellular processes that explain prevention of crc risk by high vegetable intake or higher crc risk by low vegetable intake

Human study: conclusions

- Most of the effects on the expression of genes by altering vegetable intake can be mechanistically linked to cellular processes that explain colon-cancer prevention by high vegetable intake or colon-cancer risk by low vegetable intake;
- An increased intake of vegetables resulted in down-regulation of genes promoting bioactivation of procarcinogens, and in up-regulation of genes involved in cell cycle/growth (resulting in inhibition);
- A decreased intake of vegetables resulted in down-regulation of genes inhibiting cell cycle/growth and up-regulation of genes promoting bioactivation of procarcinogens;
- For patients, genes are modulated which are involved in the late stages of colorectal cancer; for controls genes are modulated in the initiating events.

(van Breda et al. Carcinogenesis 2004;25(11):2207-16)

Conclusions & Take home messages

- Evidence of cancer prevention by high consumption of vegetables against cancers of the gastrointestinal and respiratory tract (probably) convincing → more emphasis on specific compounds;
- Vegetables contain a variety of possible anticarcinogenic compounds, which exert their anticarcinogenic effects through means of blocking and suppressing mechanisms;
- Number of studies investigating gene and/or protein expression changes in colon and lung is still limited;
- Results from expression studies show that vegetable(components) are able to modulate expression in pathways involved in carcinogenesis mostly in favour of cancer prevention;

Conclusions & Take home messages

- **Pathways modulated:**

  Colon & Lung: Apoptosis, biotransformation, cell proliferation, cell cycle, intracellular defense;

  Additional in Lung: immune response, intracellular communication;

→ **Eating a variety of vegetables and therefore a variety of anticarcinogenic compounds will trigger as many different possible anticarcinogenic mechanisms as possible, resulting in a most optimal and effective risk reduction of cancer development.**

More details:
Van Breda *et al.* Mechanisms of colorectal and lung cancer prevention by vegetables: A genomic approach. Journal of Nutritional Biochemistry, 2008:19:139-157

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QUESTIONS?