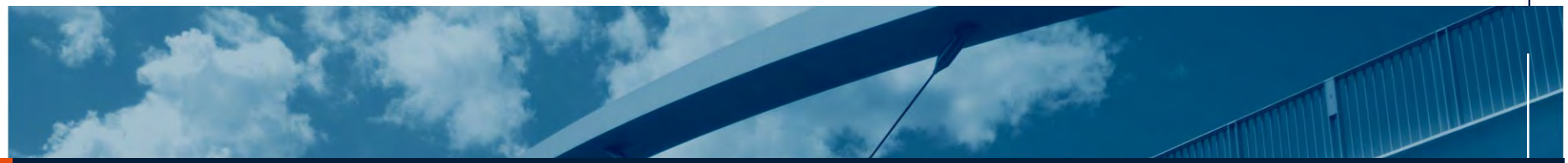
Maastricht University Leading in Learning!



The application of toxicogenomics in the risk assessment of food constituents

Prof. dr. Theo M. de Kok
Department of Toxicogenomics, Maastricht University, The Netherlands.

SKLM symposium, November 18th, 2015 Bonn Germany.

Assuring food/consumer safety

Requires toxicological evaluation of

- Food ingredients
- Whole foods
- Complete and complex diets

Traditionally making use of endpoints of toxicity of biomarkers of disease risk in the context of

- In vitro studies
- Animal experiments
- Human population or intervention studies (epidemiology)

Food safety in Europe

Food quality and safety has reached a high level as a consequence of a system of toxicological evaluations and monitoring.

Nevertheless,

- Outbreaks / incidents occur with high frequency
- New products are brought to the market
- New processing techniques are introduced
- Unperceived risk or inadequate risk assessment
  - Relatively low risk for large populations for frequent chronic diseases
  - Complex interactions between food ingredients (formation of endogenous compounds)
  - Increased risk only occurs in sub-populations (genetics – medical conditions – interactions with medication)

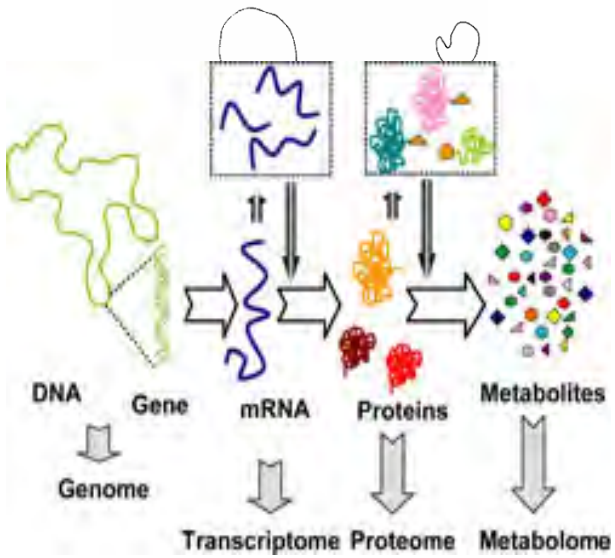
Outline

Selection of our current research topics to illustrate how the use of toxigenomics analyses can contribute to improve risk assessment :

- Safety aspects of titanium dioxide as food constituent (E171);
- Processed meat consumption (IARC group 1 carcinogen);
- Complex mixtures of bioactive phytochemicals (risk-benefit);
- Potassium bromate (E924) and ochratoxin A (challenges of multiomics analyses).

Omics approaches in human population studies

Dietary or Environmental factor



Human disease

Exposure results in specific response profiles

Genome wide molecular response profiles

These profiles can be established using a combination of high throughput omics techniques and may provide information on:

- Mode of action (molecular mechanism of toxicity);
- Type of exposure (chemical class of compounds);
- Level of exposure
- Type of toxicological effect.

These profiles may:

- be more sensitive (detection of subtle changes)
- earlier risk predictors (detection of earlier /intermediate responses)
- help to establish causality (by understanding mode of action (MoA))

Exposome - approaches

The exposome concept refers to the totality of dietary environmental exposures from conception onwards.

The internal exposome is based on measurements in biological material of complete sets of biomarkers of exposure.

Biomarkers cover a wide range of molecules, ranging from xenobiotics and their metabolites in blood (metabolomics) to covalent complexes with DNA and proteins (adductomics).

Omics refers to the measurement of a complete set of (non-) biological molecules with high-throughput techniques (Rappaport, 2010).

Omics signatures reflect the interaction between genetics and exposure characteristics (interactome); and of complex exposure profiles.

Large EU environmental health studies

- NewGeneris (FP6)
- EnviroGenomarkers (FP6)
- EXPOsOMICS (FP7)
- HELIX (FP7)
- FLEHS (Flemish Environmental Health Survey)

These projects have in common that they are using 'exposome' type of data linking various omics to exposure as well as to diseases or markers of disease
NG: not a full genomics project, first example on NOC exposure related to MN formation
EGM: has already delivered a marker for NHL
EXPOsOMICS: has no disease cases but focusses on air pollution and drinking water contaminants
FLEHS: different approach to look at combined exposure scores

Titanium dioxide = E171

- Food additive: gum, icing, candies, cookies, pastries, diary products;
- Whitening agent;
- Part nano- and part microparticles (a nanoparticle <100 nm);

Estimated intake:

- Adults: 0,82 mg/kg bw/day
- Children <10 years: 1 to 2 mg/kg bw/day

EU regulation 2013:

– E171 authorised in the Group II:

Food colours authorised at *quantum satis*

Titanium dioxide = E171

European regulation is based on:

European food safety authority 2004:

- Refers to an evaluation of titanium dioxide by the Joint WHO/FAO Expert Committee of Food Additives in 1969
- Refers to 2 toxicological studies
- Refers to unpublished study
- Refers to NCI technical support, 1979

1969: Joint WHO/FAO expert committee of Food Additives (JECFA)

- Lloyd et al. (1955): 92% in feces thus not absorbed
- Fournier et al. (1950): Absorption of titanium dioxide in rats
- Lehman et al. (1927): 2 guinea-pigs, 2 rabbits, 2 cats and 1 dog
- Vernetti et al. (1928): 3 groups of 2 dogs

Reasons for concern

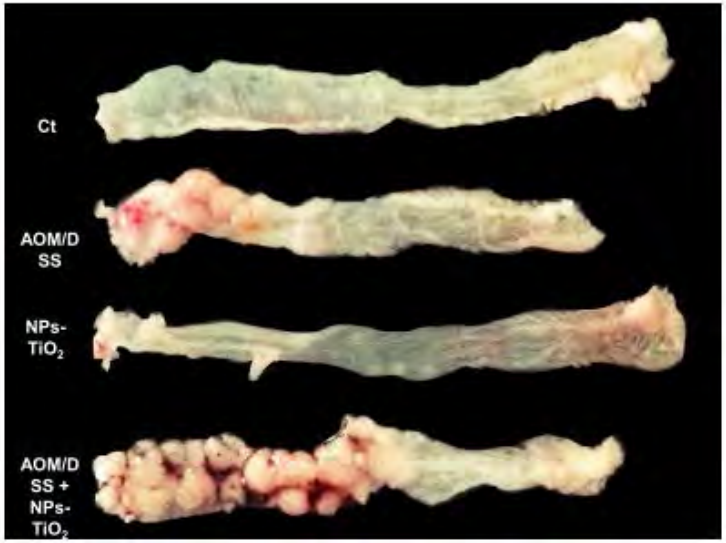
Regulations

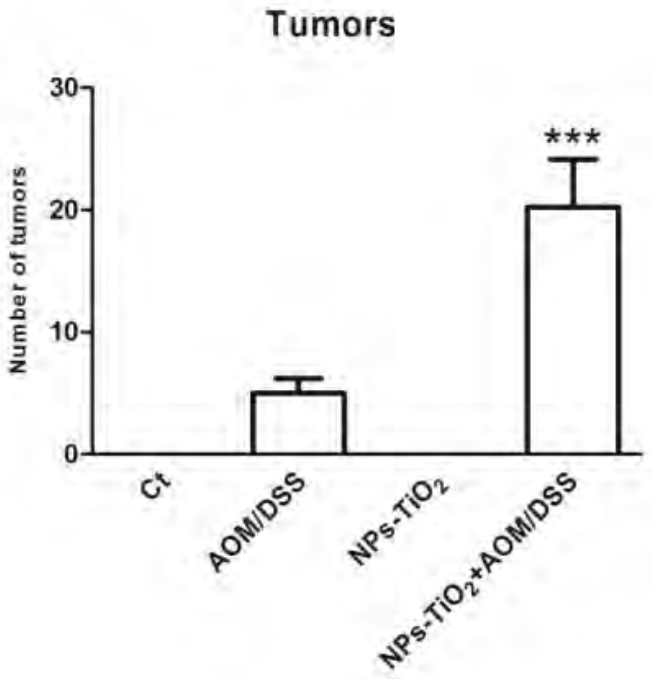
- Approved by EU since 1969
- Approved by FDA since 1966

IARC (International Agency for Research on Cancer) changed classification in 2010 from non carcinogenic to group 2B: probable carcinogen.

Recent new findings

Titanium dioxide stimulates tumor formation in mice with chemically induced colorectal cancer





I. Chirino et al 2015 Universidad Nacional Autónoma de México; Facultad de Estudios Superiores Iztacala

Free radical mechanisms involved

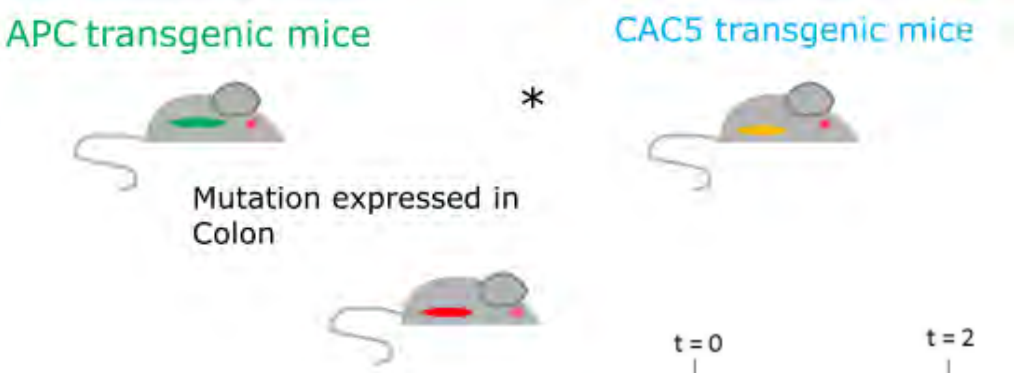
In vitro studies demonstrated that titanium dioxide:

- stimulates the production of reactive oxygen species (ESR measurements)
- Promote DNA strand breaks in colonic cells in combination with AOM

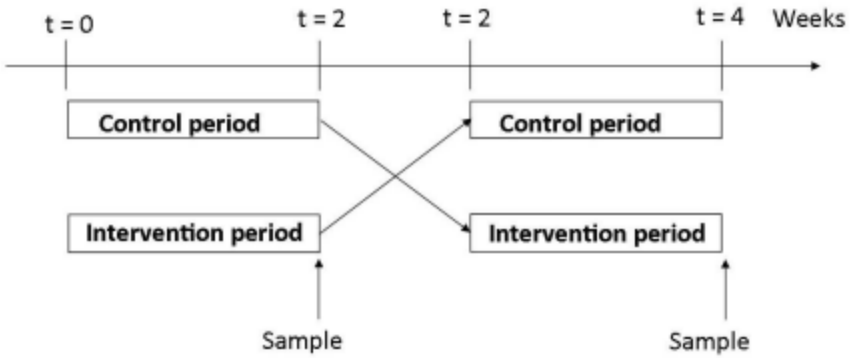
Ongoing research

To confirm that TiO2 nanoparticles can stimulate the development of colorectal cancer and to identify the molecular mechanisms, two in vivo experiments applying whole genome transcriptome analyses are being performed:

In mice:



In humans:



Processed meat consumption and cancer

International Agency for Research on Cancer

World Health Organization

PRESS RELEASE
N° 240

26 October 2015

IARC Monographs evaluate consumption of red meat and processed meat

Processed meat was classified as carcinogenic to humans (Group 1), based on sufficient evidence in humans that the consumption of processed meat causes colorectal cancer.

Processed meat consumption and cancer

Also WCRF has repeatedly indicated red and particularly processed meat (nitrite preserved) as risk factor for colon cancer.

Suggested causal food constituents:

- Pyrolysis products introduced by preparation
- Haem iron
- *N*-nitroso compounds

Haem iron induced transcriptomic changes

ORIGINAL ARTICLE

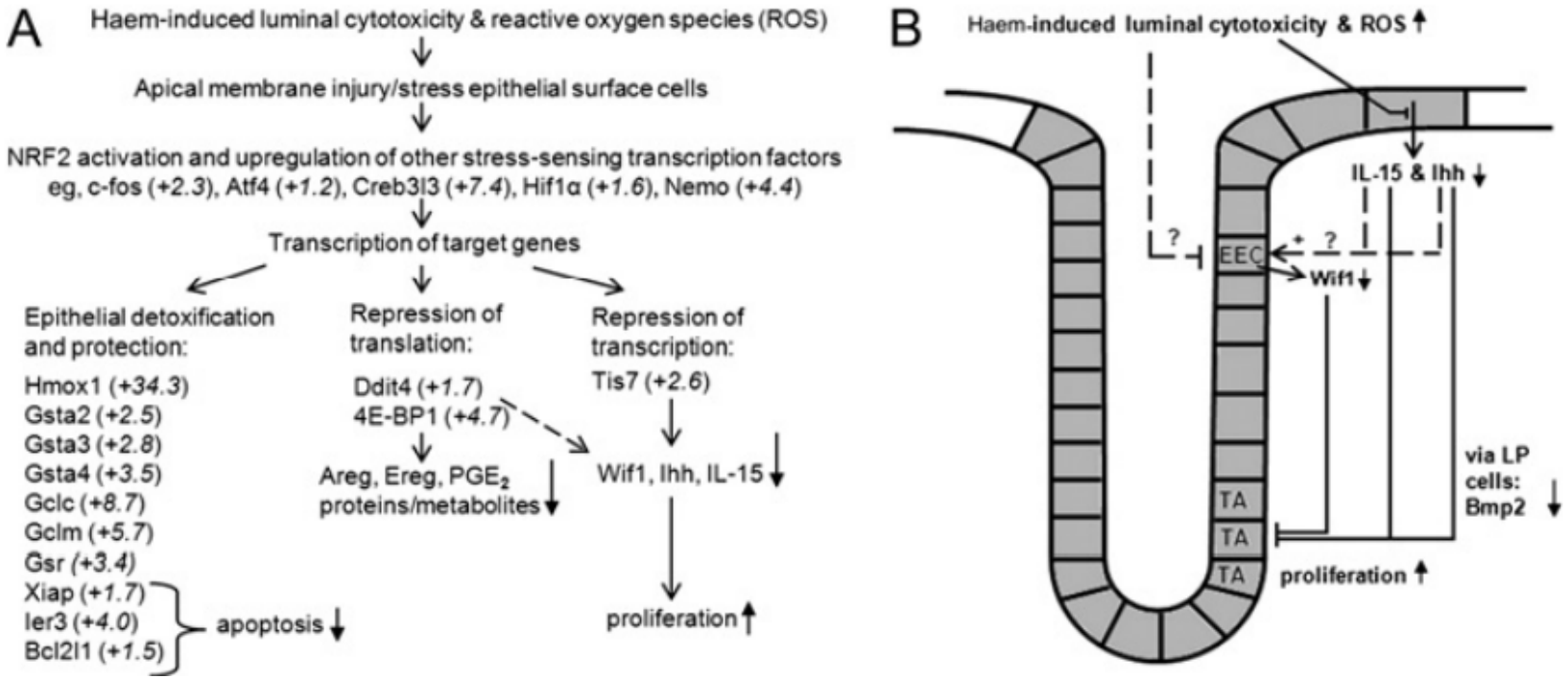
Dietary haem stimulates epithelial cell turnover by downregulating feedback inhibitors of proliferation in murine colon

Noortje IJssennagger,[1,2] Anneke Rijnierse,[1,2] Nicole de Wit,[1,3] Denise Jonker-Termont,[1,4] Jan Dekker,[1] Michael Müller,[1,2,3] Roelof van der Meer[1,2]

Gut 2012;61:1041e1049.

- Haem-fed mice showed epithelial hyperproliferation and decreased apoptosis, resulting in hyperplasia.
- Microarray analysis of the colon mucosa showed 3710 differentially expressed genes, with many involved in the cell cycle
- Signalling from the injured surface epithelium to the proliferative crypt occurs via downregulation of feedback inhibitors of proliferation (eg, Wif1, Ihh, Bmp2 and IL-15).

Haem iron induced transcriptomic changes



Model of proposed mechanism by which haem stresses surface cells and increases crypt cell proliferation. (A) Overview of main pathways changed by haem in surface cells. Fold changes in surface cells are shown in italics. (B) Model of haemmodulated signalling from surface to crypt cells.

Processed meat and *N*-nitroso compounds

N-nitrosocompounds are endogenously formed

- Typically related to consumption of red meat, not white meat;
- Requires the presence of nitrosating agent
- Genotoxic, mutagenic, alkylating properties
- Carcinogenic in animal studies

Processed meat consumption and cancer

Mutagenesis vol. 26 no. 6 pp. 753–761, 2011
Advance Access Publication 1 July 2011
doi:10.1093/mutage/ger043

Whole-genome gene expression modifications associated with nitrosamine exposure and micronucleus frequency in human blood cells

Dennie G. A. J. Hebels, Danyel G. J. Jennen, Marcel H. M. van Herwijnen, Edwin J. C. Moonen, Marie Pedersen[1], Lisbeth E. Knudsen[1], Jos C. S. Kleinjans and Theo M. C. M. de Kok

N-nitroso compound exposure-associated transcriptomic profiles are indicative of an increased risk for colorectal cancer

Dennie G.A.J. Hebels [a,*], Kirstine M. Sveje [a], Marloes C. de Kok [a], Marcel H.M. van Herwijnen [a], Gunter G.C. Kuhnle [b,c], Leopold G.J.B. Engels [d], Carla B.E.M. Vleugels-Simon [d], Wout G.N. Mares [d,e], Marieke Pierik [e], Ad A.M. Masclee [e], Jos C.S. Kleinjans [a], Theo M.C.M. de Kok [a]

Cancer Letters 309 (2011) 1–10

Selection of GeneGO pathways related to NOC excretion

Process	Pathways involved
Apoptosis and survival ⇦	Endoplasmic reticulum stress response pathway Regulation of Apoptosis by Mitochondrial Proteins Caspase cascade Cytoplasmic/mitochondrial transport of proapoptotic proteins Bid, Bmf and Bim Apoptotic TNF-family pathways
Cell cycle	Regulation of G1/S transition ⇦
Cytoskeleton remodeling	ACM3 and ACM4 in keratinocyte migration Role of PKA in cytoskeleton reorganisation ⇦ ESR1 action on cytoskeleton remodeling and cell migration
Development ⇦	Thrombopoetin signaling via JAK-STAT pathway Activation of astroglial cells proliferation by ACM3 Epidermal Growth Factor Receptor (EGFR) signaling via PIP3
G-protein signaling ⇦	RhoA regulation pathway Regulation of CDC42 activity Regulation of RAC1 activity Rac2 regulation pathway
Transcription	CREB pathway ⇦

- A genotoxic/pro-apoptotic response ⇦
- Pathways in carcinogenesis: JAK-STAT, EGFR, Rho GTPase signaling ⇦
- PKA/CREB signaling: micronucleus formation ⇦

Selection of GeneGO pathways related to MN frequency

(tertile comparison + correlation analysis)

Process	Pathways involved
Cytoskeleton remodeling	Role of PKA in cytoskeleton reorganisation ←
Development ←	Role of IL-8 in angiogenesis Endothelin-1/EDNRA signaling EDG3 signaling pathway
Signal transduction	PKA signaling ←
Transcription	CREB pathway ←

- Pathways implicated in carcinogenesis ←
- Again PKA/CREB signaling → overlap with pathways associated with NOC excretion ←

Suggests a possible link between NOC exposure and MN formation at gene expression level.

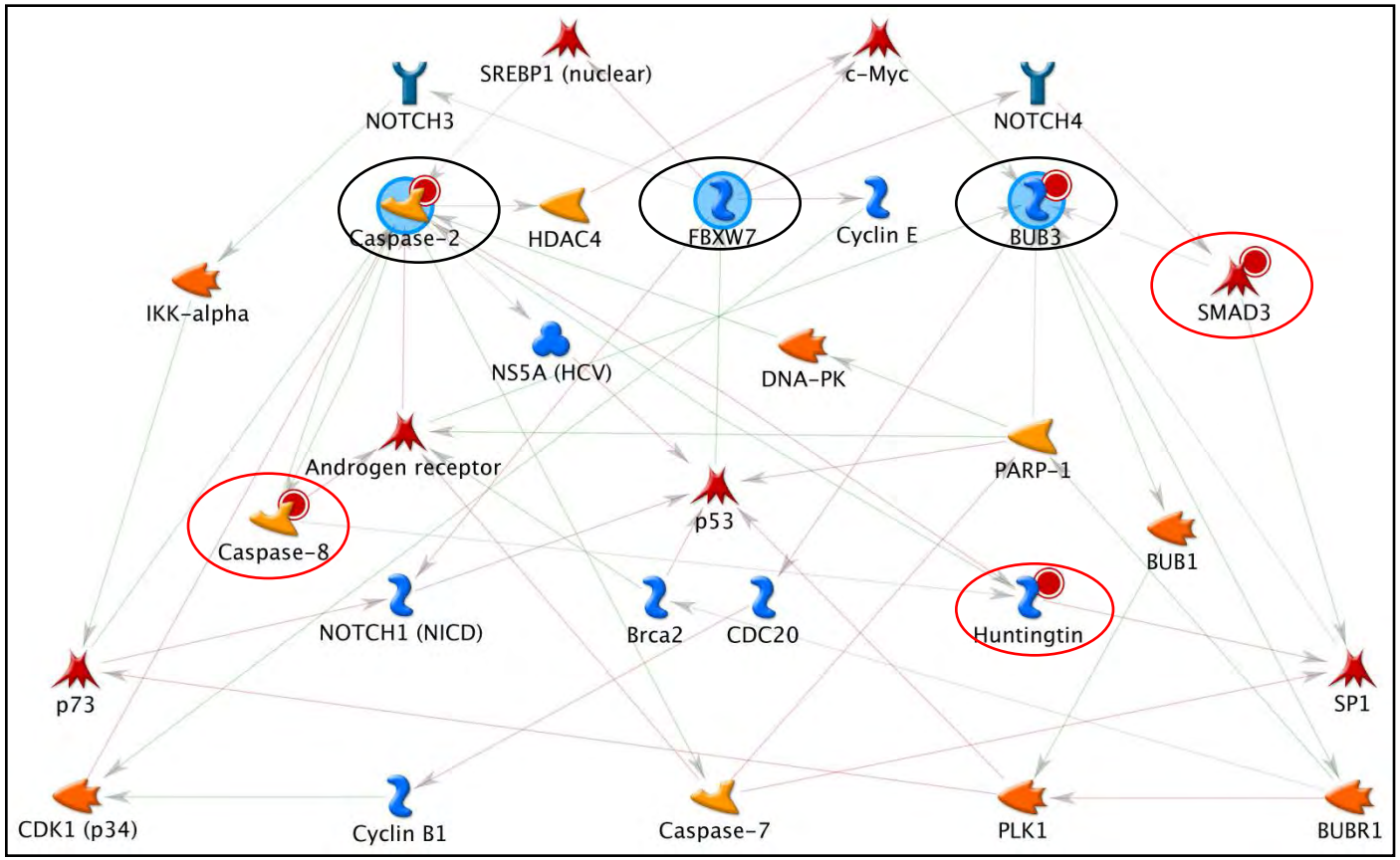
Significantly modulated and correlating genes in the NOC excretion and MN frequency associated gene sets were screened for overlap with a list of genes involved in MN formation published recently*

Three genes were found to overlap: FBXW7, BUB3, and Caspase 2

Genes in MN frequency related gene set	Genes in NOC excretion related gene set	
FBXW7	**BUB3**	**Caspase 2**
Aneuploidy; Cell cycle; DNA damage; Neoplasm/carcinoma formation; NOTCH signaling; Proteolysis	Cell cycle; Mitotic cell cycle checkpoint; Spindle assembly checkpoint	Apoptosis and survival; DNA damage; Neoplasm/carcinoma formation; Proteolysis

* Van Leeuwen et al., Transcriptomic network analysis of micronuclei-related genes: a case study, Mutagenesis 26 (1), pp. 27-32, 2011

Network analysis (MetaCore) on FBXW7, BUB3, and Caspase 2:



Three more genes in this network were found to overlap with the NOC excretion related gene set (Caspase 8, Huntingtin, SMAD3).

Potential as transcriptomic biomarker.

PHYTOME - project

PHYTOME
Phytochemicals to reduce nitrite in meat products.

Phytochemicals to produce healthy meat products!

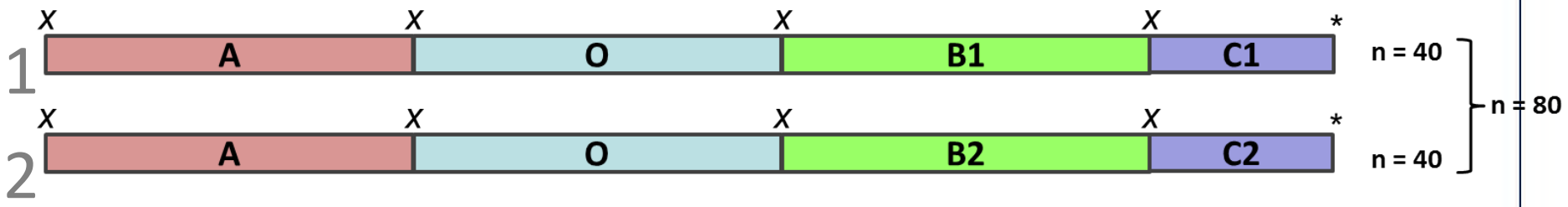
Selection of most relevant phytochemicals
Introduction of natural extracts into meat products
Reduction or elimination of nitrite from processed meats

- Evaluation of microbial safety
- Sensory quality
- Health impacts
  - reduction of NOC formation
  - changes in colonic gene expression changes
  - changes in colonic DNA damage
  - epigenetic changes (DNA methylation)

SEVENTH FRAMEWORK PROGRAMME

PHYTOME - project

A: red processed meat	O: wash-out	B: 'PHYTOME' meat	C: drinking water

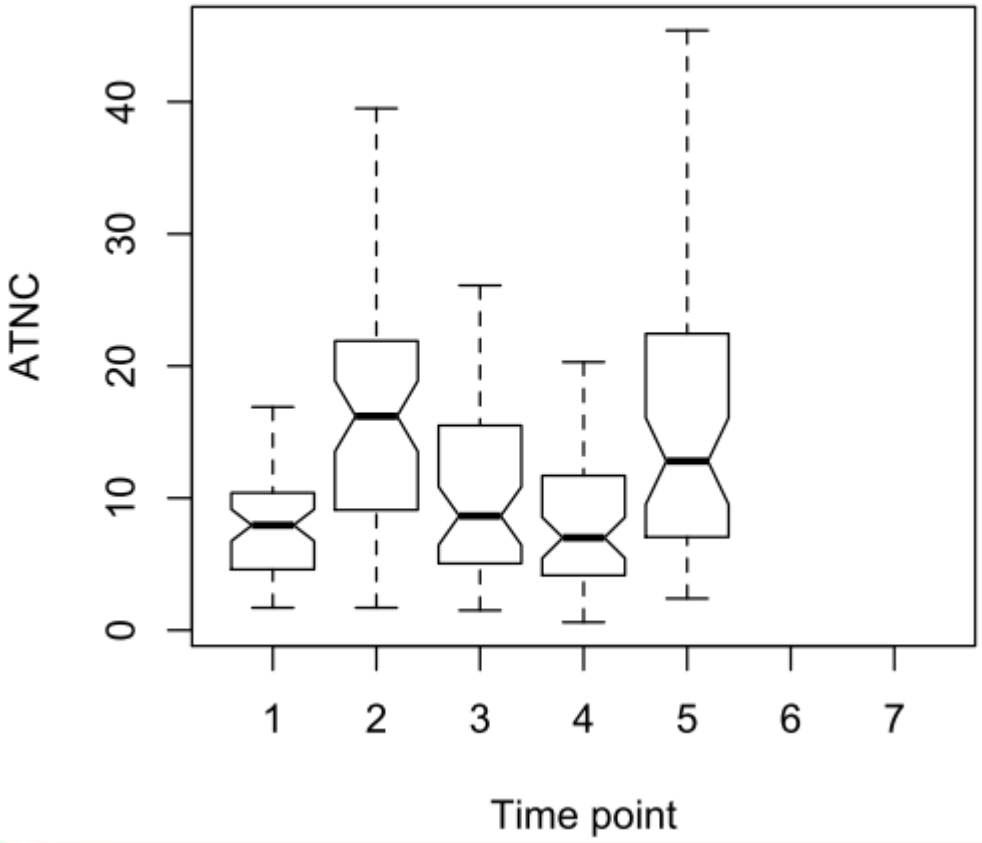


- Each intervention period = 15 days with controlled diet
- Series 1 = normal nitrite; series 2 = low nitrite
- X= Sample collection =
  - Faecal water (24h)
  - Urine (24h)
  - Blood
  - Saliva
  - Colonic biopsies

PHYTOME - project

PHYTOME
Phytochemicals to reduce nitrite in meat products.

Exposure to N-Nitroso compounds (ATNC)



- ATNC increases after consumption of processed meat
- ATNC decreases with white meat
- Meat induced ATNC formation can be (fully) reduced by addition of phytochemicals
- Drinking water nitrate stimulates ATNC (in mixed diet group)

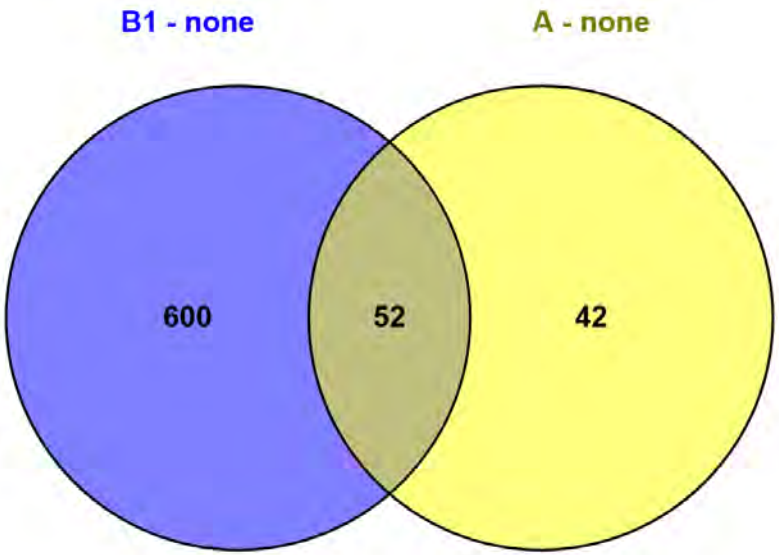
PHYTOME
Phytochemicals to reduce nitrite in meat products.

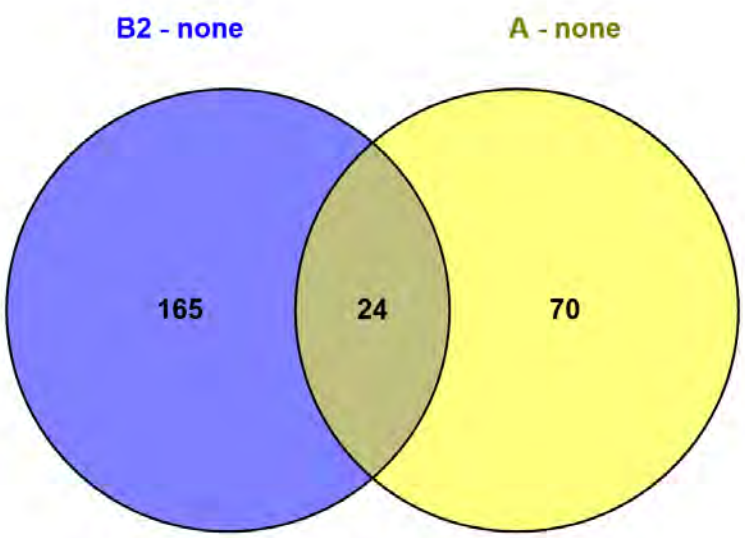
Gene expression changes:

	Red processed meat	PHYTOME meat	Overlap	Total overlap
UP	206	211	178 (43%)	329 (84%)
DOWN	184	179	151 (42%)	

Specific gene expression patterns after consumption of different types of processed meat

Comparing gene expression changes between groups:





Gene lists and biological processes show that relevant processes in relation to cancer development are differently influenced in de red meat group as compared to the PHYTOME group:

- Cell cycle control
- Regulation of APC/C activators between G1/S
- Separation of sister chromatids
- APC/C-mediated degradation of cell cycle proteins
- Gastric Cancer Network 1
- Oxidative Stress
- IL12 signaling mediated by STAT4
- Activation of APC/C and APC/C:Cdc20 mediated degradation of mitotic proteins
- cholesterol biosynthesis I

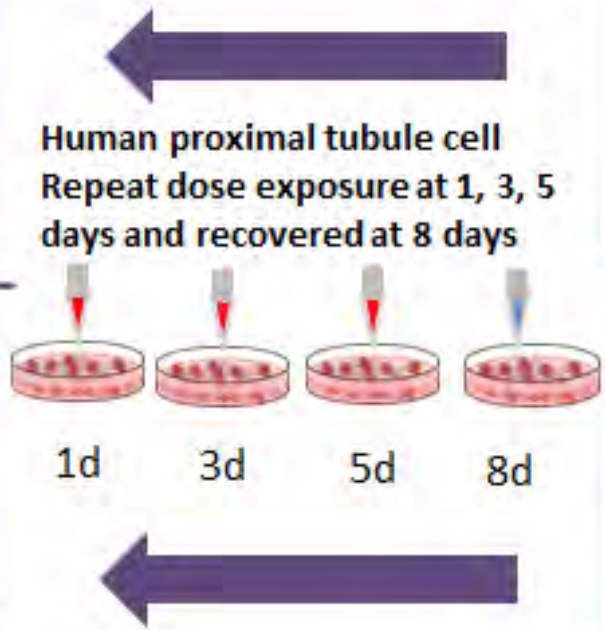
Implications for risk assessment

- Implementation of the PHYTOME concept may contribute to improved European public health by reducing NOC exposure;
- The PHYTOME project demonstrates the importance of a well balanced diet containing a wide variety of phytochemicals and a well controlled intake of dietary nitrate.
- A final statement on the carcinogenicity of processed meat: ……

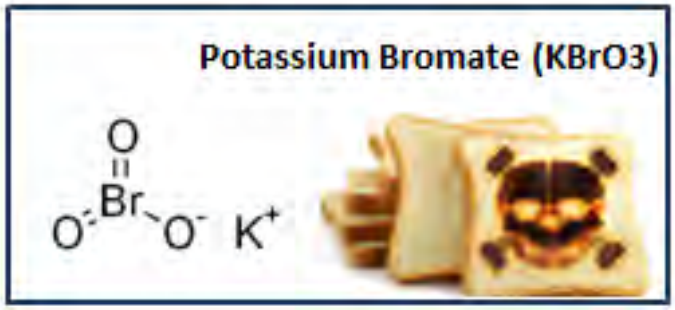
Integrating multiple "omics" analysis

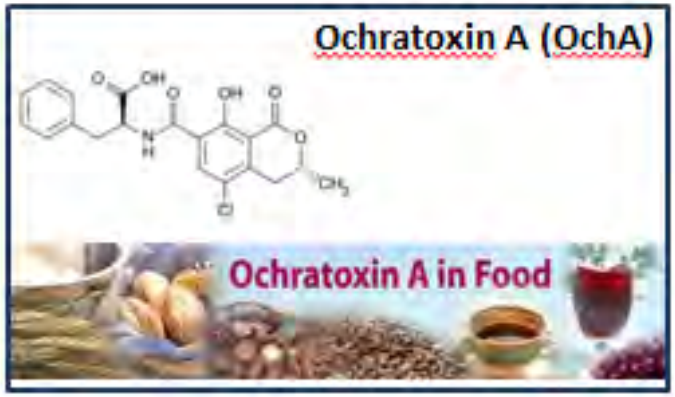
Five "omics" platforms

- Transcriptome
- MicroRNA
- Metabolome
- DNA methylation
- Histone acetylation

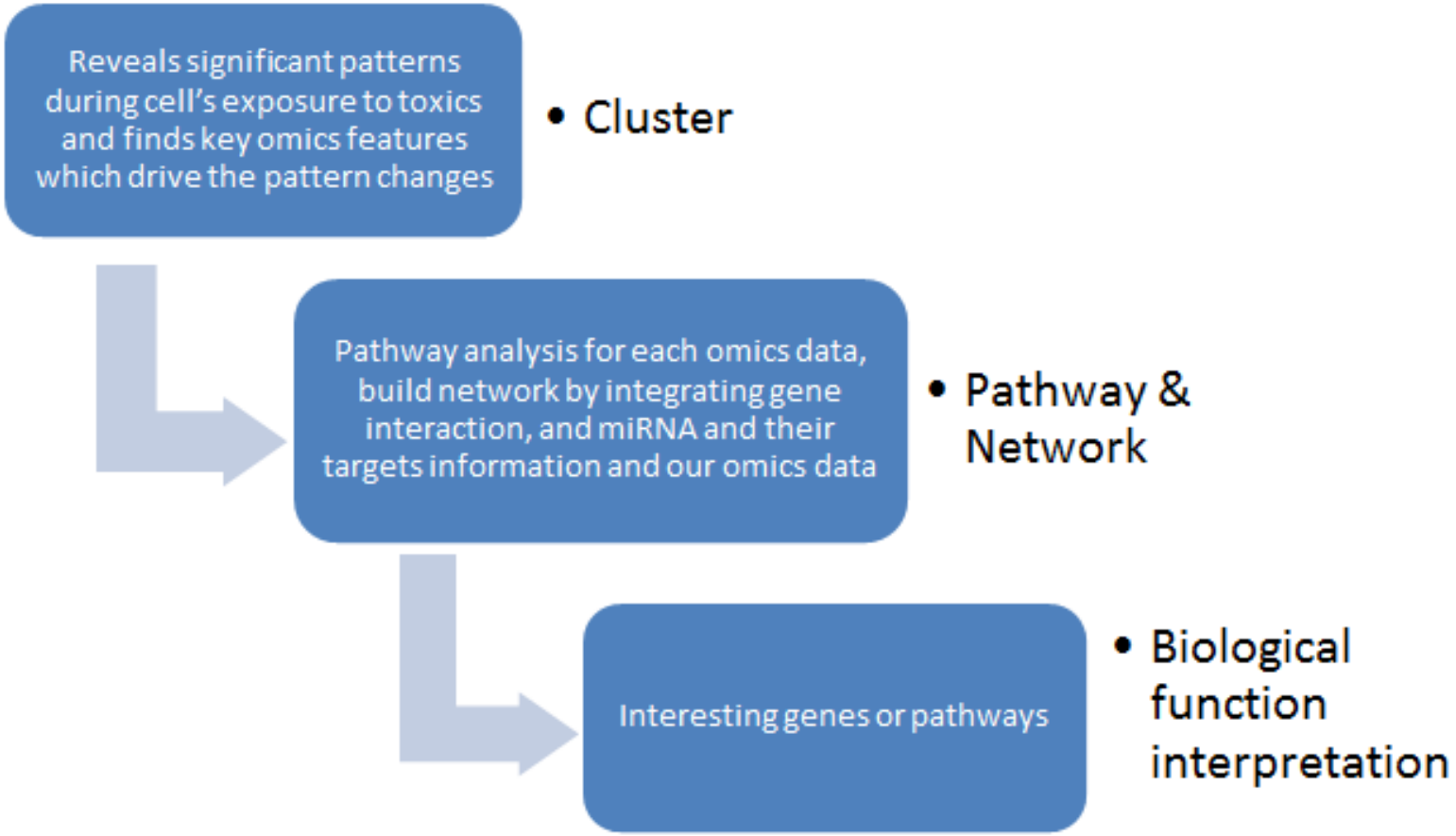


Two renal carcinogen

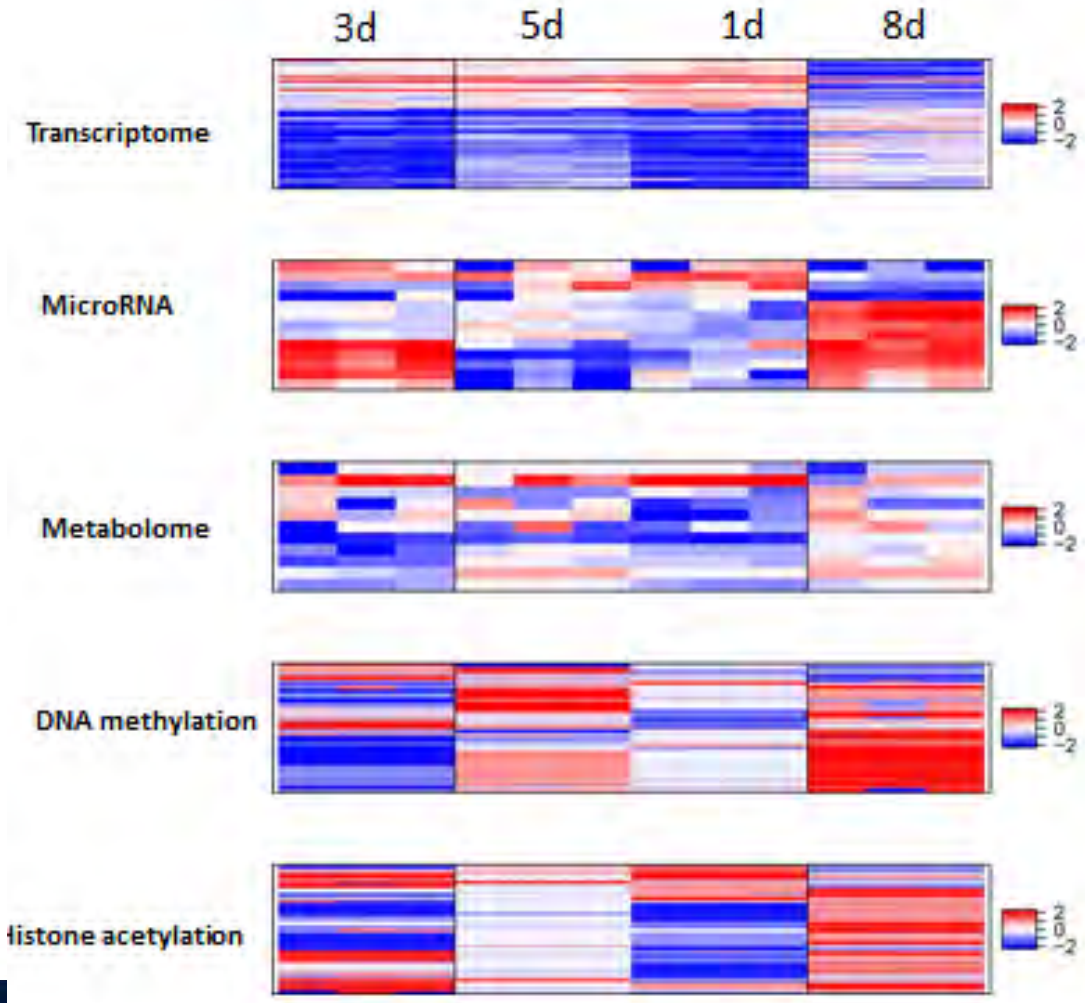


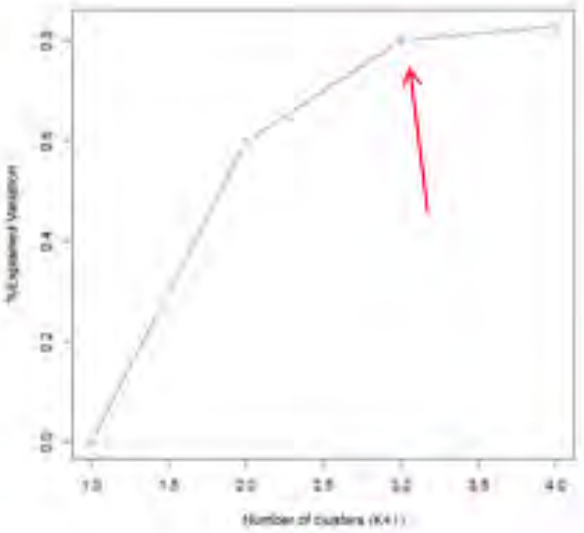


Data analysis workflow



iCluster of KBrO3 data





Cluster_membership	K=2	K=3	K=4
K1A	1	2	2
K1B	1	2	2
K1C	1	2	2
K3A	2	1	1
K3B	2	1	1
K3C	2	1	1
K5A	1	2	3
K5B	1	2	3
K5C	1	2	3
K8A	1	3	4
K8B	1	3	4
K8C	1	3	4

iCluster discriminant features

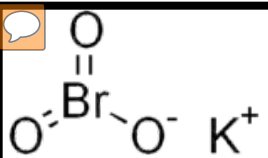
Input and output of iCluster

	KBrO3 (3 clusters)	
Data types	# Features for input	# Features after iCluster filtering prob >0.9
Transcriptome	6411	61
MicroRNA	126	13
Metabolomics	103	11
Histone acetylation	5435	534
DNA methylation	3346	322

Transcriptome pathways

q-value	pathway	source	members_input_overlap
0.000	p53 signaling pathway	KEGG	PPM1D; SESN1; FAS; CDKN1A; ZMAT3; RRM2B; GADD45A; MDM2
0.005	DNA Damage Response	Wikipathways	SESN1; GADD45A; CDKN1A; RRM2B; FAS; MDM2
0.020	miRNA Regulation of DNA Damage Response	Wikipathways	SESN1; GADD45A; CDKN1A; RRM2B; FAS; MDM2
0.026	Cytokine-cytokine receptor interaction	KEGG	CCL2; TGFB2; TNFSF10; FAS; LTB; TNFRSF10D; CX3CL1; CCL20; CXCL14; TNFRSF19
0.028	Vitamin D Receptor Pathway	Wikipathways	CYP2C9; TGFB2; SOSTDC1; GADD45A; CDKN1A; CLMN; CBS; CLDN2
0.028	HIF-1 signaling pathway	KEGG	EGLN3; HK2; CDKN1A; HMOX1; TFRC; EDN1
0.028	TNF signaling pathway	KEGG	CCL2; TRAF5; FAS; CX3CL1; EDN1; CCL20
0.034	Nuclear Receptors Meta-Pathway	Wikipathways	CYP2C9; SERPINA1; TGFB2; GSTM3; HMOX1; NQO1; CYP2C19; CCL20; CCL2; EDN2
0.034	Cell cycle	KEGG	PCNA; TGFB2; CDC27; GADD45A; CDKN1A; MDM2
0.038	Rheumatoid arthritis	KEGG	CCL2; CCL20; TGFB2; LTB; MMP1
0.039	Pathways in cancer	KEGG	EGLN3; CBLB; FZD2; TRAF5; FAS; CDKN1A; TGFB2; FGF9; WNT10A; MDM2; MMP1
0.039	Complement and Coagulation Cascades	Wikipathways	C3; SERPINA1; CFB; CLU
0.041	Trans-sulfuration and one carbon metabolism	Wikipathways	CBS; MTHFD2; TYMS
0.045	Senescence and Autophagy in Cancer	Wikipathways	PCNA; MDM2; CXCL14; CDKN1A; COL1A1
0.049	Proteoglycans in cancer	KEGG	CBLB; FZD2; FAS; CDKN1A; TGFB2; WNT10A; MDM2

Networks



biochemical_reaction
gene_regulatory_interaction
protein_interaction
\ACE
\ACE\MET
\MET
\TGX
\TGX\ACE
\TGX\MET
miRNA
Transcriptome centered network,
only the features directly connected with
TGX data are shown.
EDNRA
EDN1
EDN2
ADM
BTG2
GSTA2
hsa-miR-4281
IL27RA
IQSEC1
GSTM3
EPHA6
DIAPH3
EFEMP1
ZNF294
EPHB2
FRK
AMOT
RUNX1
EIF4G2
MITF
PPARG
DKK3
CLTCL1
ITGB3BP
HIP1
ESCO1
HIST1H3D
SUPT16H
ATRX
POLR1A
CCAR1
MBNL1
DHX9
SRSF5
HNRNPK
AGO2
GTPBP4
FTSJ3
GNL3
CAPN3
CASP4
HNRNPU
MMP2
NONO
SOX9
hsa-miR-181a-2-3p
SMAD3
DOC1
COL1A1
IGFBP3
MMP1
CCL2
MAFB
LCN2
SPOCK1
DDX17
WHSC1
SRRM2
ZFR
DDB1
TIMP4
COL7A1
RBMS3
TP53INP1
MDFIC
LGI2
ADAMTSL3
SSH1
ST14
PRH1
HPN
PSMB9
PSMB7
P3H3
KDM7A
HEG1
HIST1H1C
hsa-miR-574-3p
CDKN1A
NOTCH1
ROR1
JAK1
DYRK1A
HSPA8
HK2
ENO1
SNCB
RPS27L
TRAF5
BRD7
ATP2B4
RGS20
ADRB2
GNAZ
ATP1A4
AGTR1
EDA2R
GNAI2
NCF2
UBE3A
EHMT1
KTN1
CLU
ARRB1
NQO1
AKAP12
PDE4D
RNF40
KRT17
MDM2
PPM1J
CTDSP2
BIRC6
TBC1D1
TBC1D4
CASP1
AKT2
PRKCZ
PPM1D
PTPRA
PTP4A1
FEN1
PCNA
GRAP2
CDC27
CBLB
ASAP1
REPS2
TFRC
hsa-miR-197-3p
TNFSF10
RIPK1
FAS
TNFRSF10D
NEDD9
PTK2
PIK3R1
SSH2
CDK5
ALDOA
APEX1
RRM2B
CBS
GADD45A
ATRIP
STMN3
PIK3AP1
TGFB2
RHOU
ADSL
ATP10A
TYMS
ATP8B2
ATP9B
DHRS1
HUNK
NEK6
CIT
BRSK1
BRSK2
DAPK2
ACVRL1
DMPK
MAST4
MINK1
BCR
STK24
STK32C
WAS
MAP4K5
PTPRN
PTPN3
BATF
ATF5
ATXN1
RECQL5
MLH1
MIB1
LTB
UNC5CL
hsa-miR-1539
GALNTL4
SEMA5B
GALNT9
GALNT10
SLC17A4
SLC17A3
KRTAP10-5
PHC2
TRIM22
ISG20L1
ZNF473
RERE
CFB
C3
CYP2C19
CAPZA1
CYP2C9
CYP3A7
NEB
MMP11
SERPINA1
SVIL
COL17A1
PPL
CLMN
FBXO22
TNFRSF19
SCD5
hsa-miR-766-3p
MTHFD2
MTHFD2L
FAM83B
PHLDA3
FRMD3
hsa-miR-487b
hsa-miR-1306-3p
HMOX1
SLCO2A1
SLCO4A1
TNC
hsa-miR-132-3p
EIF5A

Omics-profiles indicative for preventive effects

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Advance Access publication June 29, 2007

Impact of multiple genetic polymorphisms on effects of a 4-week blueberry juice intervention on *ex vivo* induced lymphocytic DNA damage in human volunteers

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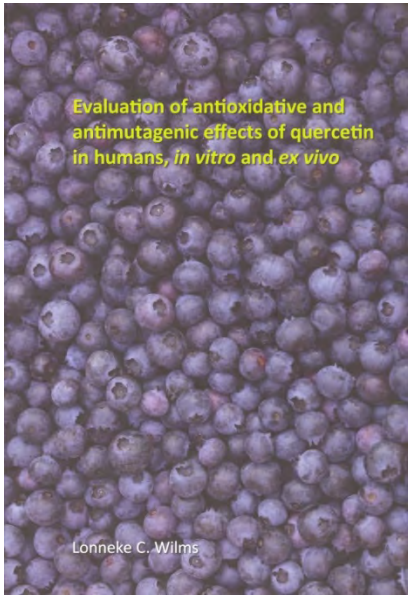


Human dietary intervention study

Hypothesis: individuals with genetic polymorphisms in genes related to quercetin metabolism, oxidative stress, BaP-metabolism and DNA repair, differ in their response to DNA protective effects of increased antioxidant intake.

Design: comparison of ex vivo induced DNA damage by H_2O_2 and BaP before and after a 4 weeks intervention with blueberry-apple juice

N = 168 (male and female);

34 biologically relevant genetic polymorphisms (e.g. NQO1*2, GSTT1, CYP2A1*1F, MPO, OGG1, …);

5-days wash out

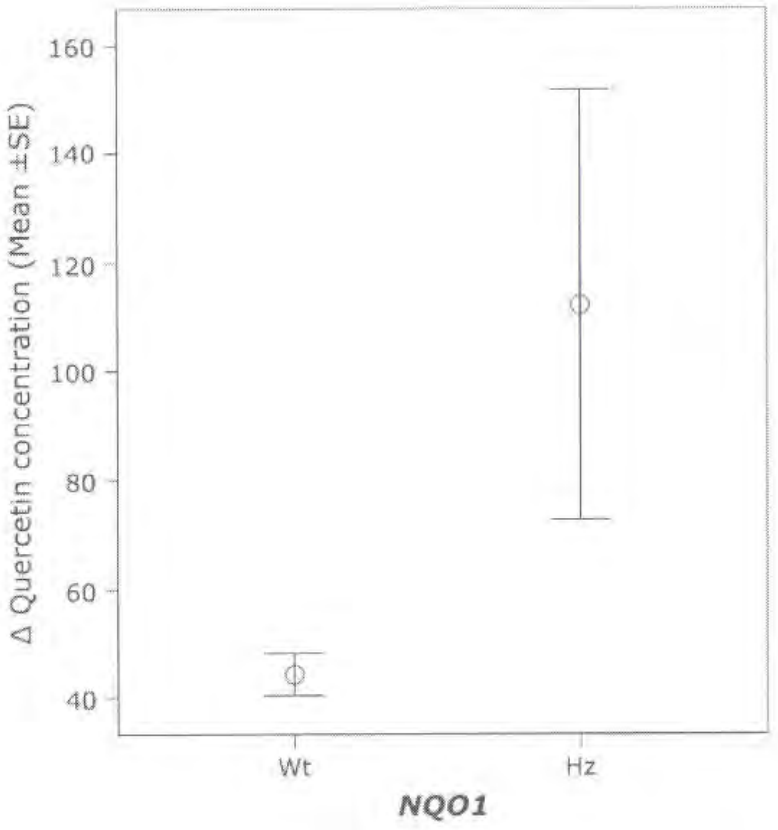
4 week intervention (97 mg Quercetin and 16 mg ascorbic acid /day);

Ex vivo induction of oxidative DNA damage (COMET) or BaP adducts (postlabeling);

Plasma quercetin concentrations

Significant increase after the intervention from 28.8 (±1.05) to 79.2 (±5.14) nM quercetin; no effect of sex and age.

NQO1*2 heterozygous subjects showed significantly larger increase than wild-types



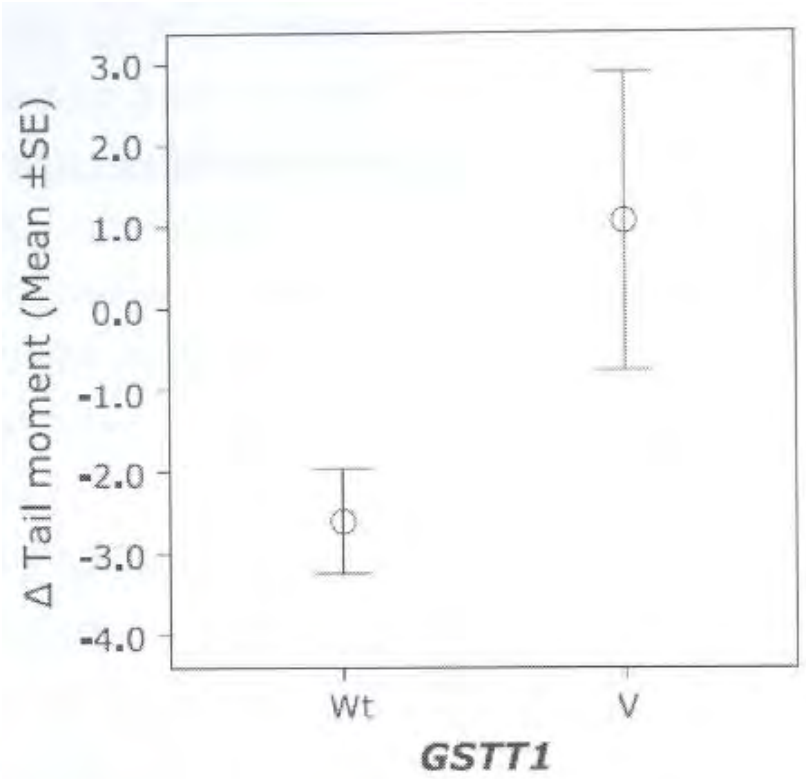
Quinone reductase (NQO1) wild-types have a higher enzyme activity and therefore probably higher metabolism of Quercetin and as a result lower free Quercetin levels in plasma. Furthermore, Quercetin may induce NQO1 gene expression and therefore even stimulates Quercetin metabolism.

NQO may also play a role in the defense against pro oxidant effects of high concentrations of Quercetin

Protection against oxidatative DNA damage

Overall a 20% reduction of oxidative DNA damage was observed;
Tail moment reduced from 9.9 (± 0.5) to 8.0 (± 0.5);
GSTT1, XRCC1 and sex were predictors of the effect.

GSTT1 wild-type show significantly higher reduction in tail moment than variants



Linking chemopreventive action to molecular pathways

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ARS OPEN Therapeutics

NEWS & VIEWS

Can Transcriptomics Provide Insight into the Chemopreventive Mechanisms of Complex Mixtures of Phytochemicals in Humans?

Simone G.J. van Breda,[1] Lonneke C. Wilms,[1] Stan Gaj,[1] Danyel G.J. Jennen,[1] Jacob J. Briedé,[1] Johannes P. Helsper,[2] Jos C.S. Kleinjans,[1] and Theo M.C.M. de Kok[1]

Whole genome gene expression analysis has been performed to identify relevant molecular pathways involved in the chemopreventive action.

Results

Several thousands of genes are differentially expressed before and after the intervention;

GSTT1 -/- show a much stronger response as compared to GSTT1 wild-types, and most genes are up-regulated:

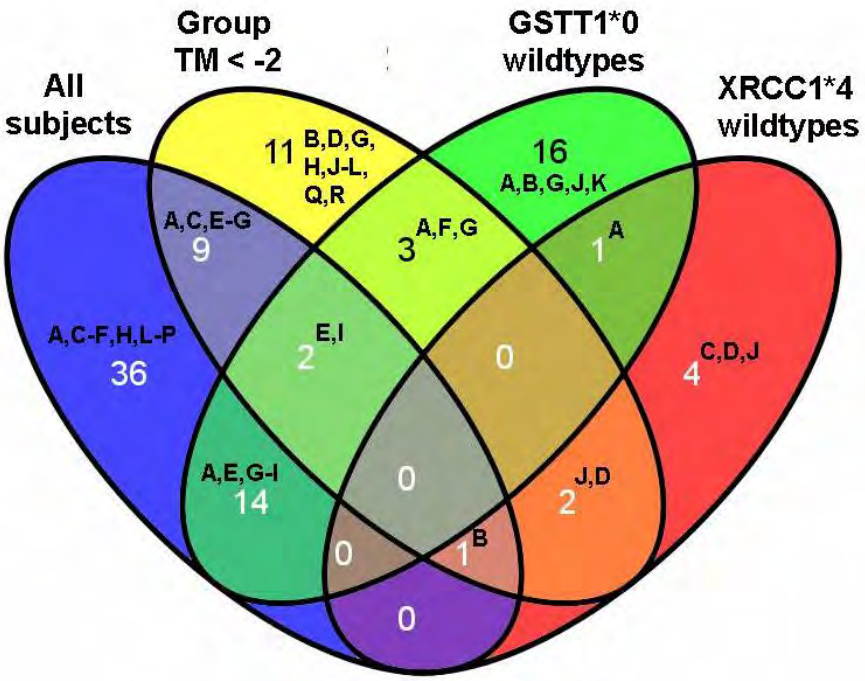
GSTT1	Total responsive genes	Up	Down
Wild-type	1091	656	435
Variant	2579	1432	1147

Results

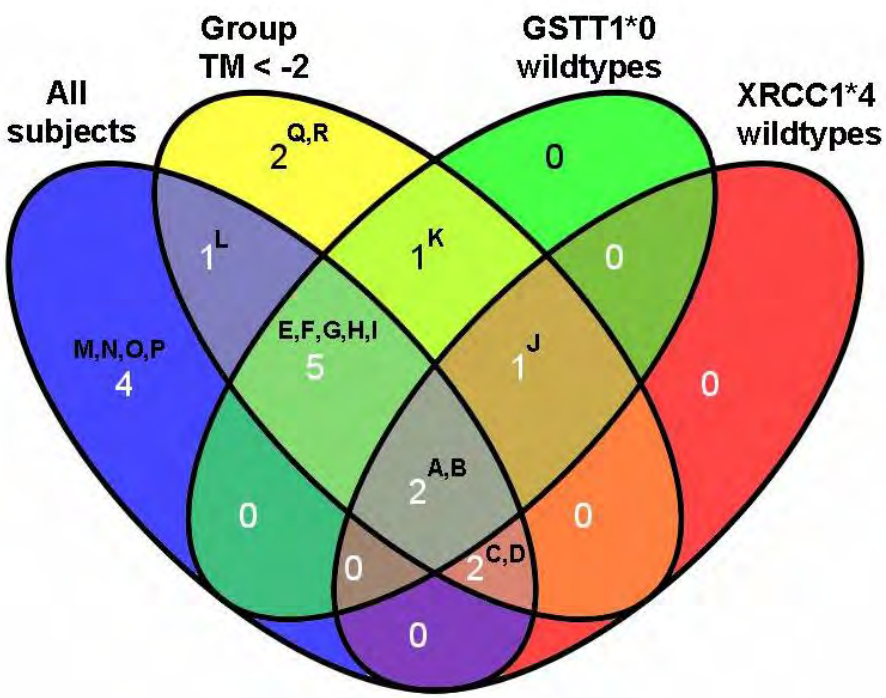
Gene group and pathway analysis to identify mechanisms involved in the chemopreventive action:

- immune response,
- cell adhesion,
- lipid metabolism,
- apoptosis

Biological pathways



Cellular processes



Legend:

- A: Immune response
- B: Cell adhesion
- C: Cystic Fibrosis
- D: Lipid metabolism
- E: Development
- F: G-protein signaling
- G: apoptosis
- H: Transport
- I: Cytoskeleton remodeling
- J: Carbohydrate metabolism
- K: Aminoacid metabolism
- L: Muscle contraction
- M: Transcription
- N: Neoplastic processes
- O: Translation
- P: Nucleotide metabolism
- Q: Neurophysiological processes
- R: Proteolysis

Cell adhesion related pathways

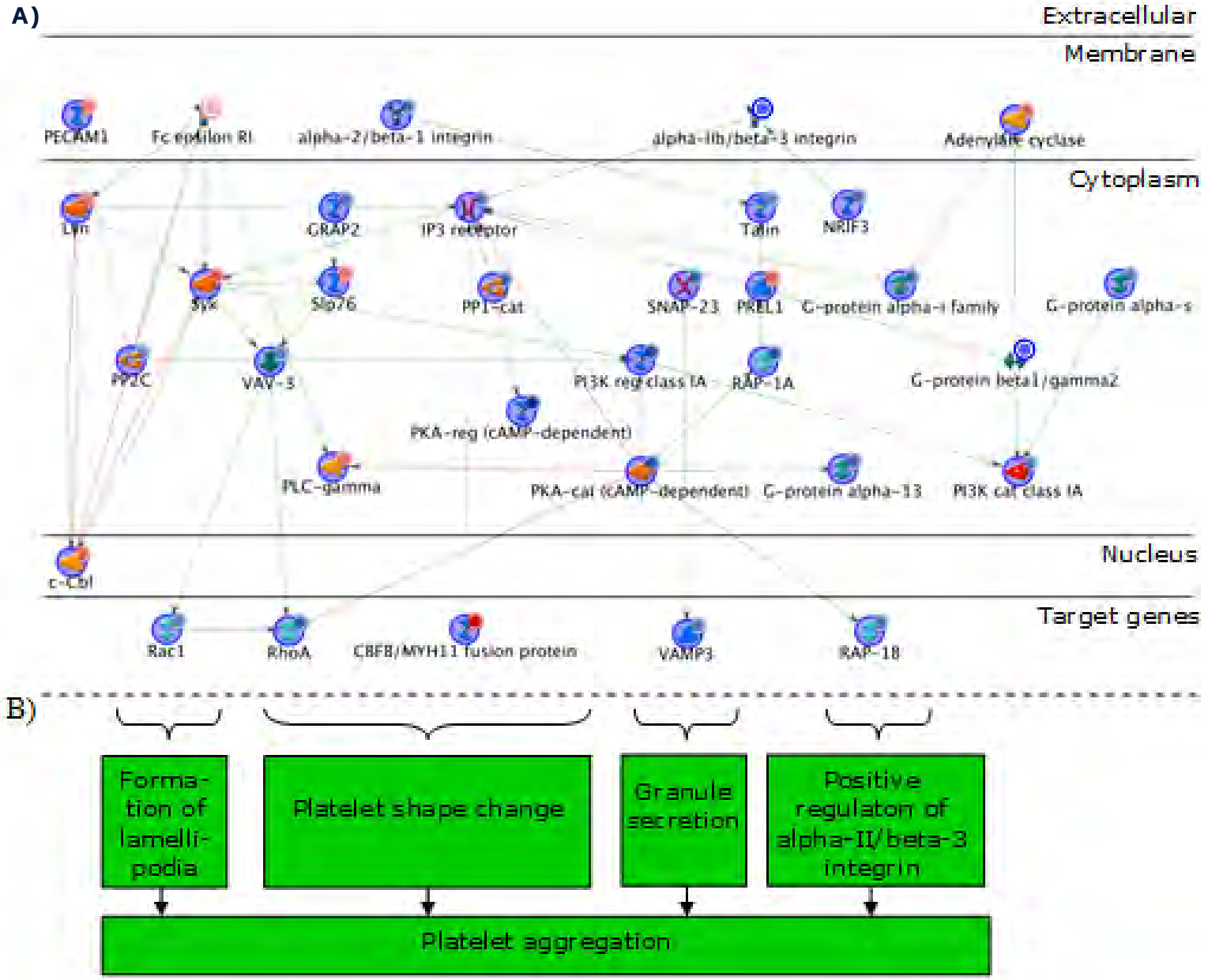


Figure above:

A) Shortest paths network pre-filtered on a sub-cellular level indicating the localization of gene expression of a selection of genes modulated in cell adhesion related pathways developed in MetaCore. Those genes of which the gene product eventually will result in a cellular effect were defined as target genes and grouped at the baseline of the network. The color of the upper right small circle at each network object indicates whether the gene was upregulated (red) or downregulated (blue).
B) Involved biological processes based on the interpretation of gene annotation and function retrieved from EntrezGene (http://www.ncbi.nlm.nih.gov/gene/) and MetaCore. The color of the square indicates whether the process will be activated (red) or deactivated (green).

Network description:
At extracellular and membrane level, several genes were modulated including the cell surface receptors *ITGA2*, *ITGB1*, and *ITGB3* which were downregulated, *PECAM1* and *FCER1G* which were upregulated, and *IP3RI* which was downregulated. In addition, the membrane bound *ADCY7* was upregulated. In the cytoplasm, a number of genes encoding for G-proteins were downregulated, among which *GNA13*, *GNAQ*, *GNAI3*, *GNAZ*, *GNA4*, *GNA5*, and *GNG11*. In addition to these genes responsible for transmitting signals from outside the cell into the cell, other genes were modulated which are part of the signaling cascades, such as the *PIK3CA* and *PIK3R1* genes (downregulated), *SYK*, *PLCG2* (both upregulated), *PRKAR2B*, *-1A*, *-2A*; and *PRKCAB* (all downregulated). Target genes which were affected include downregulation *RHOA*, *RAC1*, *VAMP3*, *RAP-1B*, and upregulation of *CBFB/MYH11 fusion protein*. Overall, these gene expression changes may lead to a reduction of several processes leading to a reduction of platelet aggregation.

Immune response related pathways

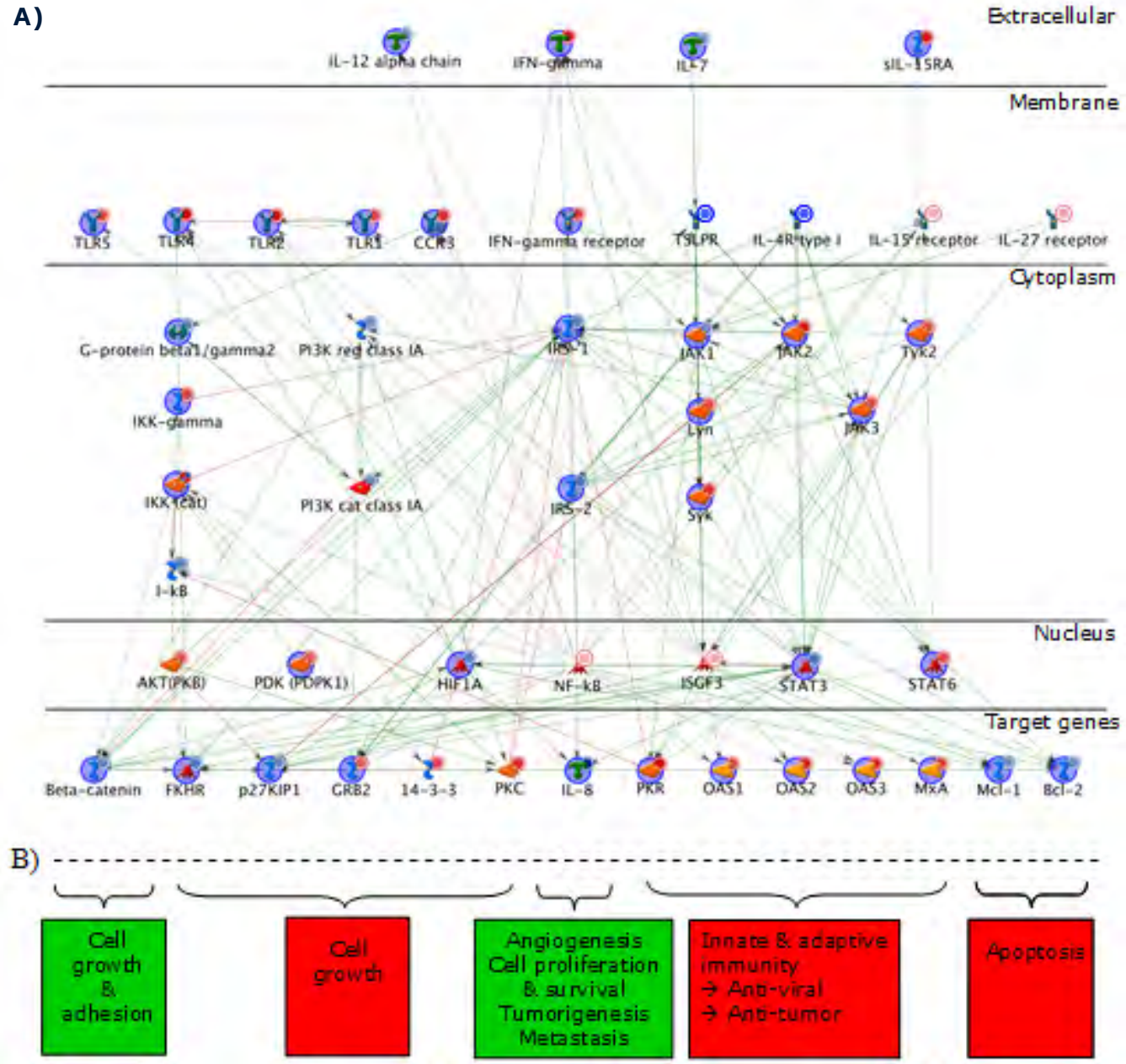


Figure above:

A) Shortest paths network pre-filtered on a sub-cellular level indicating the localization of gene expression of a selection of genes modulated in immune response related pathways developed in MetaCore. Those genes of which the gene product eventually will result in a cellular effect were defined as target genes and grouped at the baseline of the network. The color of the upper right small circle at each network object indicates whether the gene was upregulated (red) or downregulated (blue). Abbreviations can be found in the file Supplementary Figure 2 under Online Supplemental Material. Detailed information on the symbols can be found at http://www.genego.com/pdf/MC_legend.pdf.
B) Involved biological processes based on the interpretation of gene annotation and function retrieved from EntrezGene (http://www.ncbi.nlm.nih.gov/gene/) and MetaCore. The color of the square indicates whether the process will be activated (red) or deactivated (green).

Network description:
At the extracellular and membrane levels, a number of genes encoding for cytokines and receptors were found to be differentially expressed, including *IL8*, *IL12*, *IL7*, *IL7R*, *IL9R*, and *IL4R*, which were all downregulated. *IFNG*, *IFNGR*, *CCR3*, *IL27R*, *IL15R*, and *TLR1, 2, 4*, and *5* were all upregulated. These cytokines and receptors mediate a number of similar downstream signaling pathways that are critical for developmental regulation, growth control, and homeostasis. These include the JAK/STAT pathway, the PI3K/AKT pathway, and NF-κB pathway. Target genes of JAK/STAT include *BCL2* and *MCL1* which were downregulated, possibly leading to induction of apoptosis. In addition, *PKR*, *MXA*, *OAS1*, *-2*, and *-3* were upregulated, thereby initiating anti-viral and anti-tumor activities from the innate and adaptive immune system. PI3K/AKT signaling involved downregulation of target genes *FKHR*, *p27KIP1*, *14-3-3*, and *BCL2*, and upregulation of *GRB2 and PKC*. Overall, these changes may lead to an increase in cell growth. *IL8* was inhibited, which is a major target gene of the Nf-κB pathway, leading to reduced angiogenesis, cell proliferation and –survival, and to reduced tumorigenenis and metastasis. Furthermore, *HIF1A* and beta-catenin which are target genes of IL8 were downregulated, possible leading to reduced cell growth and adhesion.

Conclusions

- Some subpopulations may benefit more from the chemopreventive action of blueberry-apple juice than others.
- Gene expression analyses showed that different pathways were modulated, thereby providing insight into the underlying molecular mechanisms.
- Affected cellular processes like immune response and lipid metabolism involve free-radical mediated processes, thus influencing handling of oxidative stress.
- Specific genetic polymorphisms should be taken into account for risk-benefit analysis of phytochemicals / vegetables / fruits based on epidemiological studies

Final remarks and Perspectives

- Many studies are generating data on the internal exposome
- Several have already shown that transcriptome profiles reflect relevant gene-environment interactions;
- The relevance of evaluating gender differences and differences in genetic polymorphisms has been demonstrated;
- Validated biomarkers of exposure remain to be established;
- Data sets are becoming available for integrated omics markers, but available bioinformatics tools need to be optimized.

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