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PROCEEDINGS

Transcriptional changes in human Caco-2 colon cancer cells following exposure to a recurrent non-toxic dose of polyphenol-rich chokeberry juice

M. J. Bermúdez-Soto · M. Larrosa · J. García-Cantalejo · J. C. Espín · F. A. Tomás-Barberan · M. T. García-Conesa

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Abstract Berries and red fruits are important dietary sources of polyphenols [1]. In vitro and animal studies have demonstrated the bioavailability and the anti-proliferative and anticarcinogenic properties of these fruits or of their phenolic components [2, 3]. Consumption of berries may contribute to the reduction of colon cancer by mechanisms not yet understood. Gene expression analysis using microarrays allows for a more comprehensive study of the possible molecular mechanisms by which food or food components may prevent certain cancers of the gastrointestinal tract [4]. The aim of this research is to investigate the anti-proliferative effects of a polyphenol-rich berry juice on a human model of colon cancer cells and its association to transcriptional changes in relation to colon cancer.

Keywords Colon cancer · Caco-2 · Polyphenols · Chokeberry · *Aronia Melanocarpa* · Microarrays · Gene expression

M. J. Bermúdez-Soto · M. Larrosa · J. C. Espín · F. A. Tomás-Barberan · M. T. García-Conesa (✉)
Research Group on Quality,
Safety and Bioactivity of Plant Foods,
Food Science and Technology, CEBAS-CSIC,
Campus de Espinardo, 30100 Murcia, Spain
e-mail: mtconesa@cebas.csic.es

J. García-Cantalejo
Unidad de Genómica, Parque Científico de Madrid,
Facultad Biología, Universidad Complutense Madrid,
28040 Madrid, Spain

Methodology

We investigated the effects of a commercial chokeberry (*Aronia melanocarpa*) juice on the human model of colon cancer Caco-2 cells. In vitro digested (pepsin + pancreatin) [5] chokeberry juice was added to the cells in the culture medium at a nontoxic dose (final pH 7.5 and osmolarity 325 miliosmoles L-1 in the culture medium) 2 h a day for a 4-day period. The concentration of phenolics in the medium at time 0 of the incubation period was ~80 μM. Control cells were treated with an equivalent mix of enzymes and salts. Cells were counted using a hemacytometer and viability measured using Trypan blue dye exclusion. Results of proliferation and viability for both control and chokeberry treated Caco-2 cells are expressed as percentage of those values obtained for untreated cells. Gene expression changes were measured using microarrays (HG_U133A_2.0 human chips, Affymetrix). Transcripts that were 1.6-fold induced or repressed were selected. The changes in mRNA levels of several selected genes were further confirmed by RT-PCR.

Results and conclusions

Exposure of Caco-2 cells to pre-digested chokeberry juice resulted in inhibition of both cell proliferation (30–40%) and viability (~20%) in comparison to untreated cells. A very low proportion of genes (0.44% of total transcripts represented in the chip) were found to change in response to the treatment and most changes were in the range 1.6–2.0-fold. Resulting altered genes were categorized into several functional groups based on gene ontology search (Fatigo, GEPAS 1.1, Bioinformatics Unit, CNIO; SOURCE, Genetics Department, Stanford University) and available

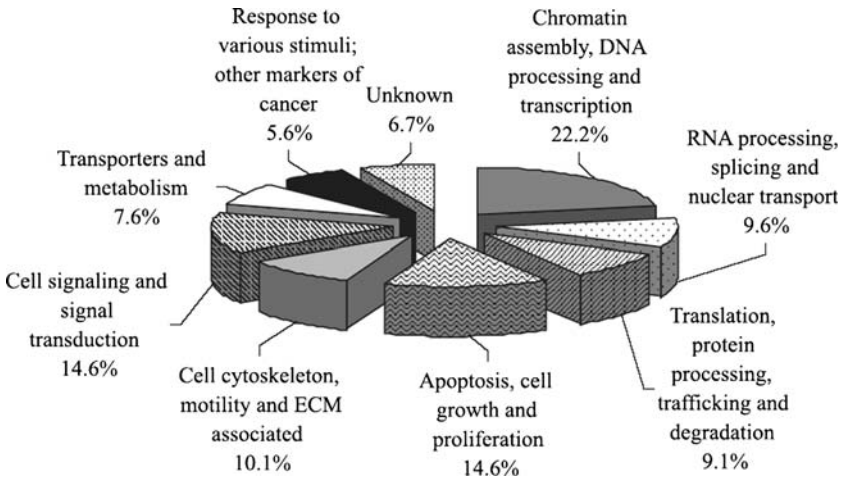

Fig. 1 Diagram of the percentage of altered genes categorized in functional groups in Caco-2 colon cancer cells after treatment with a non-toxic recurrent dose of a chokeberry juice rich in polyphenols

literature (Fig.1): (1) DNA processing and transcription, (2) cell signalling and signal transduction, (3) apoptosis, cell growth, proliferation, (4) cell cytoskeleton (5) RNA processing, (6) translation and protein processing, (7) transporters, metabolism, (8) other markers and response to various stimuli. A group of genes were also categorized as "unknown" function. Among the responsive genes we detected changes in several genes that have been reported to be related to colon carcinogenesis, tumour migration and cell proliferation. Changes in the expression levels of some of these genes were further confirmed by RT-PCR (Table 1). In conclusion, polyphenol-rich chokeberry exhibited anti-proliferative effects in Caco-2 cancer colon cells. Inhibition of the cells proliferation by chokeberry juice may be associated to the modulation of transcription of specific genes such as: (1) upregulation of tumor suppressors (CEACAM1 [6] and BMP2 [7]) and (2) down-regulation of genes related to tumor invasion and metastasis

Table 1 List of genes related to colon cancer with altered expression levels after treatment with chokeberry juice

Accession number	Gene name	Gene symbol	Affyx[a]	RT-PCR[a]	Biological process involved in
NM_001712	Carcinoembryonic antigen-related cell adhesion molecule 1	CEACAM1	+2.1	+2.6	Reduced expression is a major event in colorectal cancer. Tumor suppressor involved in cell–cell adhesion that regulates apoptosis in colon epithelium
BC000478	Heat shock 70 kDa protein 9B (mortalin 2)	HSPA9B	–2.0	n.d.	Control of cell proliferation and cellular aging. Over-expressed in colorectal adenocarcinoma
NM_022975	Fibroblast growth factor receptor 2	FGFR2	–4.6	–1.8	Receptor for fibroblast growth factor implicated in tumor growth and invasion (colon carcinoma)
NM_001200	Bone morphogenetic protein 2	BMP2	+1.6	+2.4	Belongs to the transforming growth factor β family. It acts as tumor suppressor promoting apoptosis in mature colonic epithelial cells and inhibiting proliferation
NM_005228	Epidermal growth factor receptor	EGFR	+1.8	n.d.	Receptor of the EGF family members. Involved in the control of cell growth and differentiation of colon cancer
NM_002823	Prothymosin, alpha (gene sequence 28)	PTMA	–1.8	n.d.	Nuclear protein involved in cell proliferation. Highly expressed in human colorectal cancer
NM_005242	Coagulation factor II (thrombin) receptor-like 1	F2RL1	+2.0	n.d.	Protease activated receptor involved in the control proliferation in colon cancer. Transmembrane receptor coupled to G protein
D13889	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	ID1	–1.7	n.d.	Involved in cell growth regulation and tumorigenesis. Up-regulated in colorectal cancer
NM_002624	Prefoldin 5	PFDN5	–1.6	n.d.	Chaperone protein. It may target actin and tubulin. It represses transcritption of c-myc and it is related to colon cancer

Table 1 continued

Accession number	Gene name	Gene symbol	Affyx[a]	RT-PCR[a]	Biological process involved in
NM_002961	S100 calcium binding protein A4 (metastasin)	S100A4	–2.5	–1.9	Associated with metastatic capacity of cancer cells. May function in motility, invasion and tubulin polymerisation. Significant pronostic marker of colorectal carcinoma
NM_000900	Matrix Gla protein	MGP	+1.9	n.d.	Extracellular matrix protein. It may be involved in cell differentiation and tumor progression. It is downregulated in colorectal cancer
NM_002203	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	ITGA2	+3.0	+2.6	Cell-surface protein that participates in cell adhesion (hemidesmosomes). Role in cell proliferation and migration. Involved in colorectal cancer
NM_004949	Desmocollin 2	DSC2	–1.8	n.d.	Required for cell adhesion and desmosome formation. May be involved in colorectal carcinoma metastasis
AJ224869	Chemokine (C-X-C motif) receptor 4	CXCR4	+1.7	n.d.	G-protein coupled receptor. May play a role in colon cancer and metastasis
NM_002184	Interleukin 6 signal transducer (oncostatin M receptor)	IL6ST	+3.0	–1.2	Part of the cytokine receptor complex linked to signal transduction. May be involved in cell growth and proliferation. Expressed in colorectal cancer
NM_000846	Glutathione S-transferase A2	GSTA2	–1.7	n.d.	Detoxification of electrophilic compounds by conjugation with glutathione. Overexpression in colon cancer cells protects against cell cycle arrest and apoptosis

n.d. not determined

[a] Fold up-/down-regulation

(FGFR2 [8] and S100A4 [9]). We are currently further investigating the changes in these genes and proteins.

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