Common beans and their non-digestible fraction: antitumor activities- An overview

Haydé A. Vergara-Castañeda[1], B. Dave Oomah[2], Guadalupe Loarca-Piña[3], and Rocio Campos-Vega[3, 4]*

[1]Nucitec, S. A. de C. V., Departamento de Investigación, Comerciantes 15-3, Colonia Peñuelas, Queretaro, 76148, México.

[2]National Bioproducts and Bioprocesses Program, Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.

[3]Programa de Posgrado en Alimentos del Centro de la República (PROPAC), Research and Graduate Studies in Food Science, School of Chemistry, Universidad Autónoma de Querétaro, Querétaro, Qro, México.

[4]Kellogg de México, Carretera al Campo Militar, San Antonio de la Punta, Querétaro, Qro., México.

E-mails: Guadalupe Loarca-Piña: loarca@uaq.mx; B. Dave oomah: oomahd@agr.gc.ca; Haydé A Vergara-Castañeda: hvergara@nucitec.com.

* Corresponding author: telephone (442) 211 1400 Ext 1720, chio_cve@yahoo.com.mx and rocio.campos@kellogg.com

The US Department of Agriculture's My Pyramid guidelines introduced a near doubling of the dietary recommendations for vegetables including dry beans; an important food staple in many traditional diets. Populations with high legume (peas, beans, lentils) consumption have a low risk of cancer and chronic degenerative diseases. Common beans (*Phaseolus vulgaris* L.) are known as a rich reliable source of non-digested compounds like fiber, phenolics, peptides and phytochemicals associated with health benefits. Emerging evidence indicates that common bean consumption is associated with reduced cancer risk in human populations, inhibiting carcinogenesis in animal models and inducing cell cycle arrest and apoptosis in cell cultures. Fiber may reduce the risk of premature death from all causes, while the whole non-digestible fraction from common beans has demonstrated anti- proliferative and apoptosis induction on *in vitro* and *in vivo* colon cancer. The mechanisms responsible for this apparently protective role may include gene-nutrient interactions and modulation of protein expression. This paper reviews the bioactivity and health potential of beans on tumor inhibition, highlighting studies involving functional compounds, mainly non-digestible fraction, that modulate genes and proteins, thereby unravelling its chemopreventive role against the development of cancer.

Keywords: common beans; chemoprevention; antitumor activities; non digestible fraction; bioactive compounds.

Introduction

Dry common bean (*Phaseolus vulgaris* L.) is a legume widely consumed throughout the world and is considered a good source of high protein (23%), complex carbohydrates, dietary fiber and some vitamins and minerals. The consumption of dry common beans has been associated with reduced risk of various chronic, metabolic and degenerative diseases such as cancer, obesity, diabetes and cardiovascular diseases. In addition to these nutritional components, common beans are rich in several phytochemicals with potential health benefits such as polyphenolic compounds, lectins and trypsin inhibitors (Beninger and Hosfield, 2003).

Carbohydrates constitute the main fraction of beans (55 to 65% dry weight on average) with polysaccharides as the major constituents, and small but significant amounts of oligosaccharides. The carbohydrate fraction of legumes include monosaccharides (ribose, glucose, galactose, and fructose), disaccharides (sucrose and maltose), the soluble sugar fraction, and oligosaccharides of the raffinose family (raffinose, stachyose, and verbascose), besides cellulose, lignin, pectin, galactose, arabinose, mucosa and xylose, that according to some authors, have to be grouped under the concept of "dietery fiber" or of "non digestible carbohydrates". Dietary fibre or cell wall material content in the cotyledon of legume seed is comparatively lower than that of the testa. The carbohydrate-oligosaccharide fraction of beans includes starch, soluble sugars and dietary fiber. Many health benefits are attributed to these components of bean seeds. The American Association of Cereal Chemists (AACC) defines dietary fiber (DF) as "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine" and it consists of polysaccharides (such as cellulose, hemicellulose and pectins), oligosaccharides, lignin and associated plant substances (Campos-Vega et al., 2011).

Emerging evidence indicates that common bean is associated with reduced cancer risk in human populations and rodent carcinogenesis models. Epidemiological and preclinical studies evaluating colon cancer (Bobe et al., 2008; Mentor-Marcel et al., 2009; Hangen and Bennink, 2002; Lanza et al., 2006), prostate cancer (Mills et al., 1989; Kolonel et al., 2000), and mammary cancer (Thompson et al., 2012) have lent further support for an inverse relationship between bean consumption and the development of cancer. However, the mechanism of action implicated on this health benefits are limited.

Common bean & non digestible fraction

Colorectal cancer (CRC) is one of the most common neoplasms afflicting industrialized societies (Ferlay et al., 2010). Both genetic and environmental exposures have been implicated in the etiology of CRC, and it has been estimated that up to 75% of cases may be preventable by adequate diets and regular exercise (Armstrong and Doll, 1975; Doll and Peto, 1981; WCRF and

AICR, 2007). Additionally, populations consuming higher intakes of legumes (peas, beans, lentils, peanuts) have lower risk and mortality from CRC (Lanza et al., 2006; Correa, 1981).

Epidemiological evidence suggests a protective role of dietary fiber against CRC (Dahm et al., 2010). However, the presence of pulse phytochemicals such as phenolic compounds (condensed tannins, flavonoids, and anthocyanins), total dietary fiber (TDF) (soluble and insoluble), lectins, unsaturated fatty acids, phytic acid, trypsin inhibitors, and other secondary metabolites have been positively associated with the prevention and/or reduction of chronic degenerative diseases (Bazzano et al., 2001; Beninger and Hosfield 2003; Waldecker et al., 2008; Campos-Vega et al., 2010a). Some of these substances (nondigestible fraction [NDF] and phenolic compounds) may reach the colon to be fermented by the microflora, producing mainly short-chain fatty acids (SCFAs) such as acetic, propionic, and butyric acids (Delzenne et al., 2003). The latter is a 4-carbon fatty acid that has been studied as a chemopreventive agent because of its inhibition against tumor cell proliferation, induction of apoptosis leading to a more differentiated phenotype (Sengupta et al., 2006), thus reducing the risk of developing CRC.

Several studies have demonstrated the potential of bean-based diets to inhibit AOM-induced colon cancer in animal model (Feregrino-Pérez et al., 2008; Vergara-Castañeda et al., 2010; Vergara-Castañeda et al., 2012; Bennink and Om, 1998; Bennink et al., 1999; Hangen and Bennink, 2002; Rondini and Bennink, 2012). For example, a polysaccharide extract of black bean cultivar (cv.) Negro 8025 reduced aberrant crypt foci development in azoxymethane (AOM)-induced rats and regulated the expression of β-catenin, p53, p21, Rb, Bax and caspase-3 (Casp3) genes involved in cell proliferation, cellular arrest and apoptosis (Feregrino-Pérez et al., 2008). Vergara-Castañeda et al., (2010) demonstrated that cooked bean and NDF from Bayo Madero also provide direct chemoprotection against early stage of azoxymethane (AOM)-induced colon cancer in rats by suppressing aberrant crypt foci (ACF) and reducing β-glucuronidase activity, an enzyme with glucuronide conjugate hydrolyzing ability that may release active carcinogenic metabolites in the intestinal lumen. Furthermore, the transcriptional effects of the NDF from common bean cv. Bayo Madero were investigated on the gene expression profile in the distal colon tissue of Tp53 signal transduction in an *in vivo* model of early-stage colon cancer to elucidate the molecular mechanism involved in chemoprevention (Vergara-Castañeda et al., 2012). Significant differences were detected in seventy-two genes of the Tp53-mediated signalling pathway involved in apoptosis, cell-cycle regulation and arrest, inhibition of proliferation and inflammation, and DNA repair, as represented in **Figure 1**. Tp53, Gadd45a, Cdkn1a, and Bax were highly expressed (9·3-, 18·3-, 5·5- and 3·5-fold, respectively) in the NDF + AOM group, whereas Cdc25c, Ccne2, E2f1 and Bcl2 were significantly suppressed (29·2-, 22·6-, 218·4- and 23·5-fold, respectively), among other genes, compared with the AOM group, suggesting that chemoprevention of aberrant crypt foci results from a combination of cell-cycle arrest in G1/S and G2/M phases and cell death by apoptotic induction.

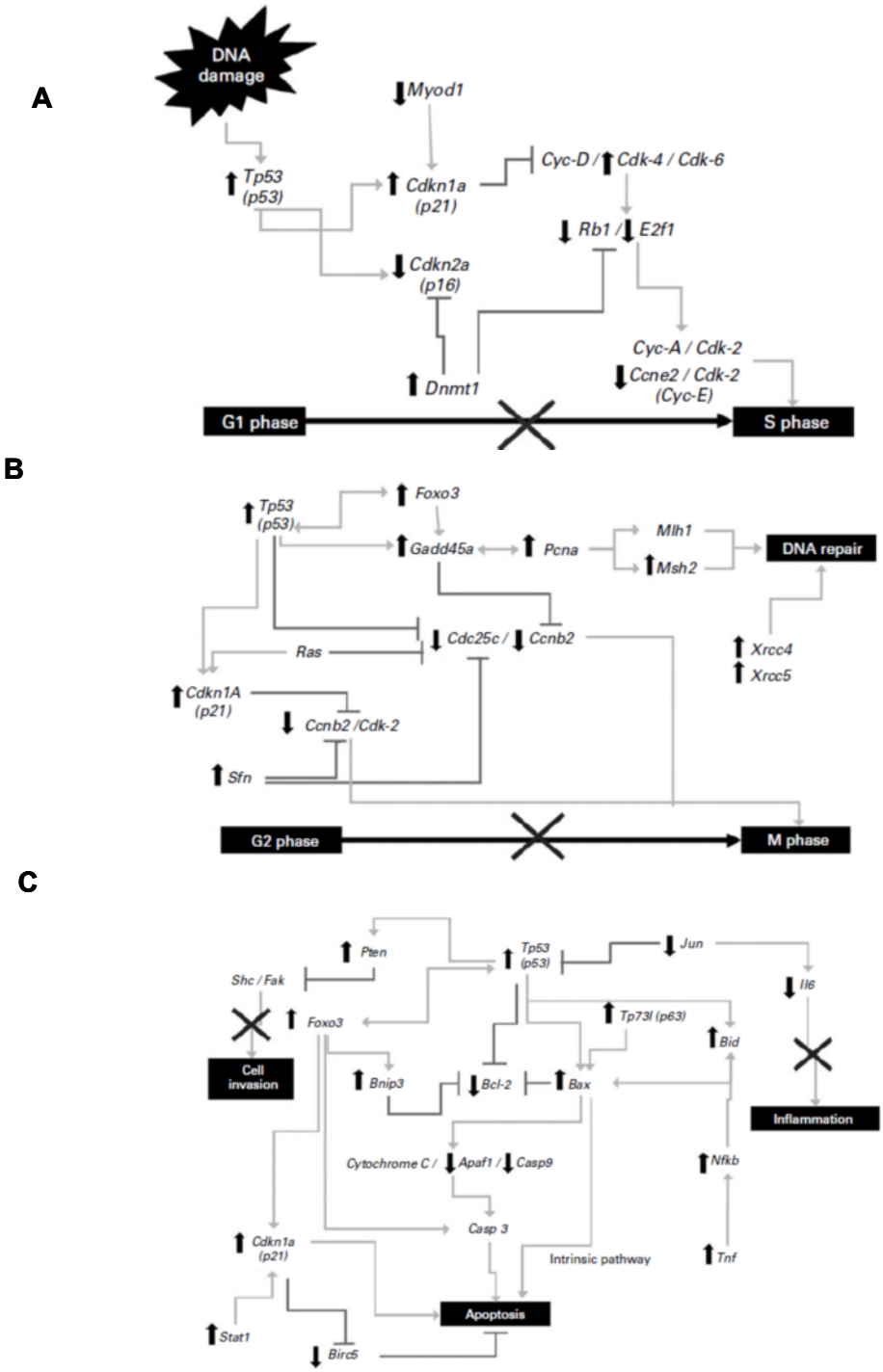

Figure 1. A) Changes in gene expression in the G1/S cell-cycle phase. B) Changes in gene expression in the G2/M cell-cycle phase and DNA repair. C) Changes in gene expression in apoptosis and inflammatory pathways. Symbols indicate up-regulation (↑) and down-regulation

(↓) in mRNA expression as derived from array analysis, and signalling pathway interruption (X). Adapted from Vergara-Castañeda et al., (2012).

A research group has demonstrated that black bean (BB) and soy flour (SF)-based diets inhibit azoxymethane (AOM)-induced colon cancer and suggests beans inhibit colon carcinogenesis by modulating cellular kinetics and reducing inflammation, potentially by preserving mucosal barrier function (Bennink and Om, 1998; Bennink et al., 1999; Hangen and Bennink, 2002; Rondini and Bennink, 2012). AOM treatment induced a number of genes involved in immunity, including several MHC II-associated antigens and innate defense genes (RatNP-3, Lyz2, Pla2g2a) (Rondini and Bennink, 2012). BB- and SF-fed rats exhibited higher expression of genes involved in energy metabolism and water and sodium absorption and lower expression of innate (RatNP-3, Pla2g2a, Tlr4, Dmbt1) and cell cycle-associated (Cdc2, Ccnb1, Top2a) genes. Genes involved in the extracellular matrix (Col1a1, Fn1) and innate immunity (RatNP-3, Pla2g2a) were induced by AOM in all diets, but to a lower extent in bean-fed animals (**Figure 2**).

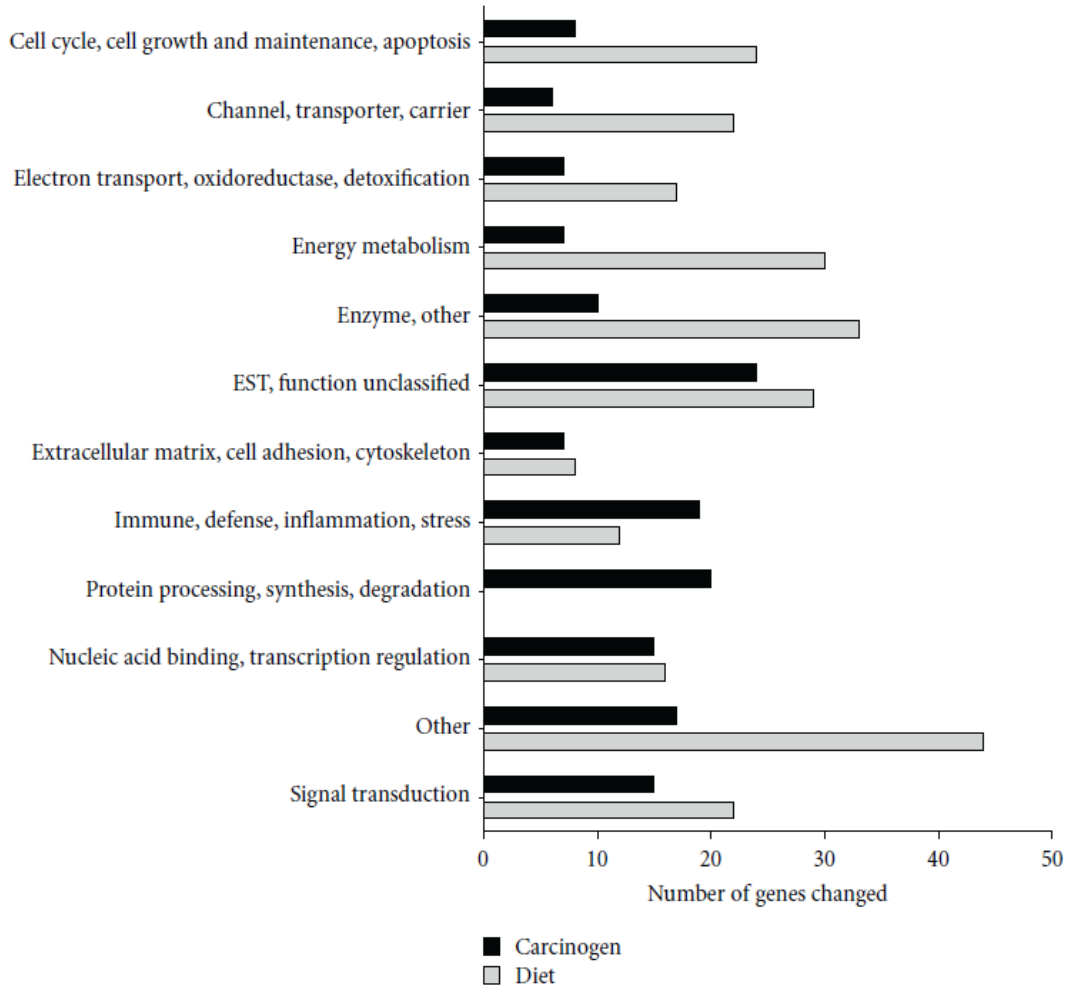

Figure 2. Functional classification of genes significantly altered by carcinogen (AOM) and by dietary treatment in the distal colon mucosa of rats detected by microarrays. A total of 155 genes were altered by carcinogen (AOM) and 257 by dietary treatment (Control versus BB versus SF, $P<0.05$). From Rondini and Bennink (2012).

The protection by common beans, NDF and SCFAs has been studied using *in vitro* cell culture, widely used to assess the effect by which a substance induces differentiation and inhibits the survival of transformed cells. HT-29 cell line has been used as a model to investigate the mechanism of some protective compounds (Cruz-Bravo et al., 2011; Campos-Vega et al., 2012b). In this regard, Campos-Vega et al., (2009) reported that common bean is an excellent source of NDF that can be fermented in the colon and produce SCFAs, compounds previously reported to exert health benefits (**Table 1**).

Table 1. Amount of short-chain fatty acids (SCFAs) (mmol/L) in fermented extract of NDF from cooked common bean seeds.

	6 h			12 h			24 h		
Sample	**Acetate**	**Propionate**	**Butyrate**	**Acetate**	**Propionate**	**Butyrate**	**Acetate**	**Propionate**	**Butyrate**
Negro 8025	39 ± 0.3^{Ax}	8 ± 0.0^{Ay}	8 ± 0.3^{Ay}	42 ± 1.0^{Ax}	9 ± 0.6^{Ay}	10 ± 0.6^{By}	51 ± 0.7^{Bx}	12 ± 0.7^{By}	15 ± 0.7^{Cz}
Bayo Madero	36 ± 0.3^{Ax}	6 ± 0.6^{Ay}	7 ± 0.3^{Ay}	44 ± 0.3^{Bx}	9 ± 0.7^{By}	10 ± 0.6^{By}	48 ± 1.0^{Cx}	12 ± 0.3^{Cy}	15 ± 0.3^{Cz}
Pinto Durango	32 ± 0.7^{Ax}	7 ± 0.3^{Ay}	6 ± 0.0^{Az}	36 ± 0.3^{Ax}	9 ± 0.6^{Ay}	8 ± 0.0^{Bz}	48 ± 0.6^{Bx}	9 ± 0.0^{Ay}	13 ± 0.3^{Cy}
Azufrado Higuera	33 ± 0.6^{Ax}	8 ± 0.0^{Ay}	6 ± 0.3^{Ay}	37 ± 0.3^{Bx}	11 ± 0.3^{By}	9 ± 0.3^{Ay}	39 ± 0.3^{Bx}	14 ± 0.3^{Cy}	14 ± 0.3^{Bz}
Rafinose (control)	14 ± 0.5^{Ay}	1 ± 0.1^{Ax}	1 ± 0.1^{Ax}	28 ± 2.7^{By}	6 ± 1.0^{Ax}	5 ± 0.7^{Bx}	30 ± 0.6^{By}	8 ± 0.4^{Bx}	2 ± 0.2^{Ax}

Results are the average of 3 independent experiments ± SEM. A,B,C Means in the same column for cultivars and SCFA with different letters are different ($P < 0.05$). x,y,z Means in the same row for hours with different small letters are different ($P < 0.05$). From Campos-Vega et al., (2009).

Molecular changes involved in p53 pathway in HT-29 cells have been evaluated by PCR array, after 24 h exposure to *in vitro* fermented NDF (cv. Bayo Madero), with human gut flora (FE-hgf) (Campos-Vega et al., 2010a). Significant differences were detected in 72 of 84 human p53-mediated signal transduction response genes involved in apoptosis, cell cycle and cell proliferation showing significant expression changes. Apoptosis genes, SIAH1, PRKCA and negative regulation of the cell cycle gene MSH2 were the highest up-regulated genes (30.5-, 18.4- and 9.8-fold, respectively), whereas cell cycle genes CHEK1 and GADD45A were markedly down regulated (21.4- and 9.1-fold, respectively) (**Figure 3**). They demonstrate that common beans and or/it´s NDF modulate gene expression profiles in HT-29 cells, providing insight about the mechanism underlying its overall chemoprotective function against colon carcinogenesis. **Figure 4** summarizes genes modulation from NDF *in vitro* and *in vivo*.

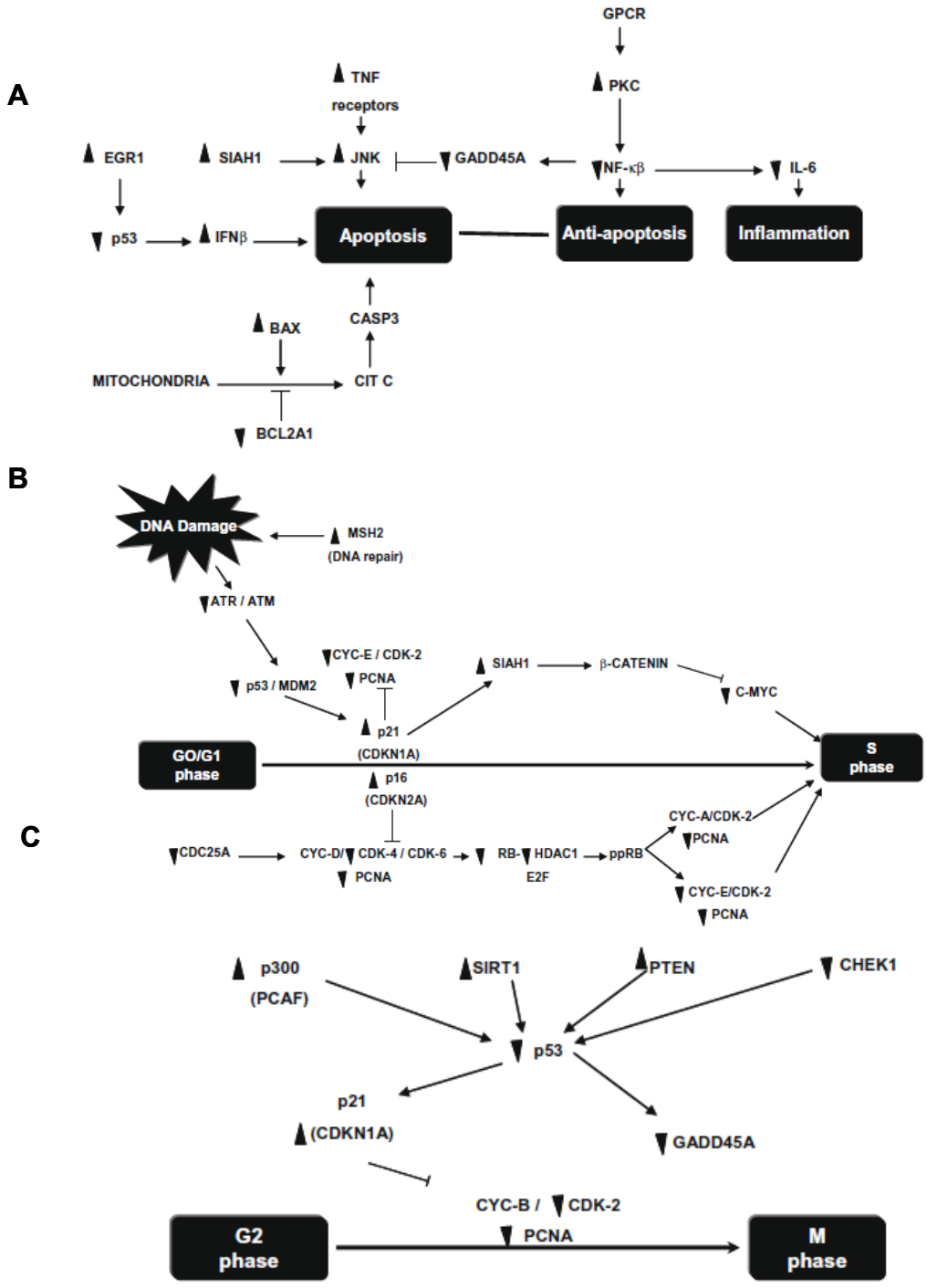

Figure 3. A) Changes in gene expression in apoptosis and inflammatory pathways. B) Changes in gene expression in G1/S cell cycle phase. C) Changes in gene expression in G2/M cell cycle

phase. Symbols indicate up-regulation (▲) or down regulation (▼) in mRNA expression as derived from array analysis. Adapted from Campos-Vega et al., (2010a).

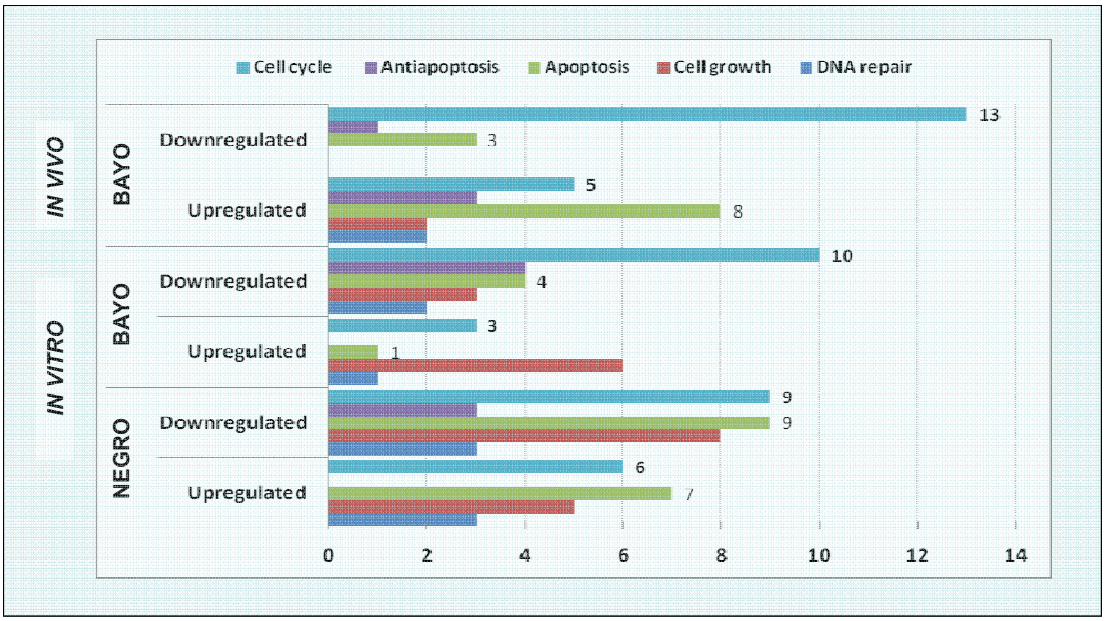

Figure 4. Modulates genes from NDF *in vitro* and *in vivo*. (Adapted from Campos-Vega et al., 2010a; Vergara-Castañeda et al., 2012; Cruz-Bravo et al., unpublished data).

Cruz-Bravo et al., (2011) extended previous study with cultivar Negro 8025 (Feregrino-Pérez et al., 2008) combining biochemical analysis with experiments designed to assess the effect of *in vitro* fermented NDF (FNDF) on the survival of the colon adenocarcinoma HT-29 cells. The results showed that FNDF inhibits HT-29 cell survival in a time and concentration-dependent manner [the lethal concentration 50 (LC_{50}) was 13.63% FNDF (equivalent to 7.36, 0.33, and 3.31 mmol of acetic, propionic, and butyric acids, respectively)]. DNA fragmentation, an apoptosis indicator, was detected by the TdT-mediated dUTP nick end labeling method (TUNEL) in cells treated with the LC50-FNDF and a synthetic mixture of SCFAs mimicking LC_{50}-FNDF (**Figure 5**).

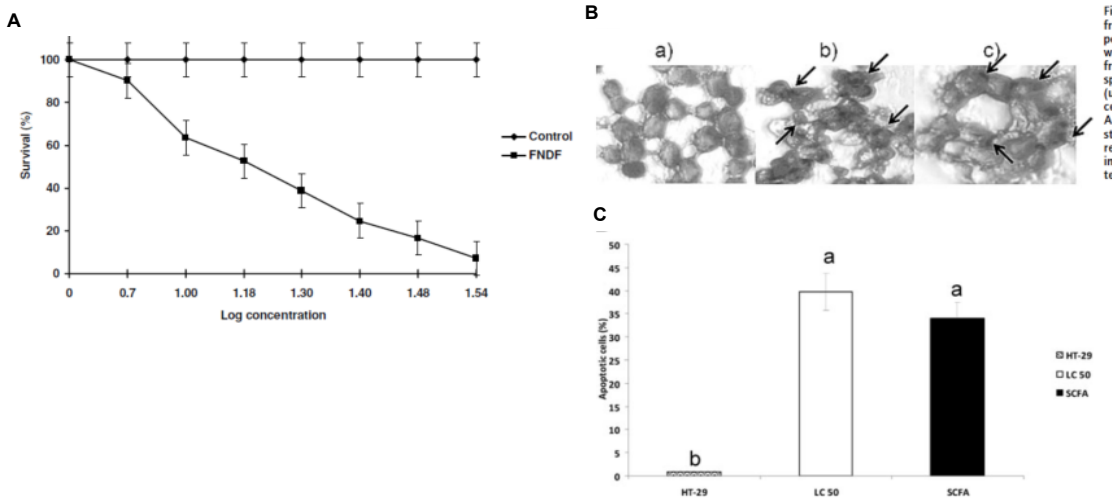

Figure 5. A) Concentration-response curve of FNDF on HT-29 cells survival. Each value represents the average of 2 independent experiments ± SD. B) Effect of FNDF on DNA fragmentation. Results are expressed as the percentage of apoptotic cells. B) Apoptotic cells were identified by TUNEL technique DNA fragmentation that can be observed as brown spots (indicated by an arrow). (a) Control (untreated HT-29 cells), (b) LC_{50}-FNDF treated cells, and (c) LC_{50}-SCFA treated cells. C) Apoptotic cells (%). Results are the mean ± standard error of 2 experiments with 2 repetitions each. Different letters by sample indicate significant difference P = 0.05, Tukey's test. Adapted from Cruz-Bravo et al., (2011).

Recently, Campos-Vega et al., (2012a), suggested that FNDF from common beans can elicit beneficial chemoprotective effects in colon cancer by modulating protein expression in HT-29 cells. FNDF inhibited HT-29 cell growth and modulated protein expression associated with apoptosis, cell cycle arrest, and proliferation (**Figure 6**), as well as morphological changes linked to apoptosis evaluated by TUNEL and hematoxylin and eosin stains, confirming previous results on gene expression.

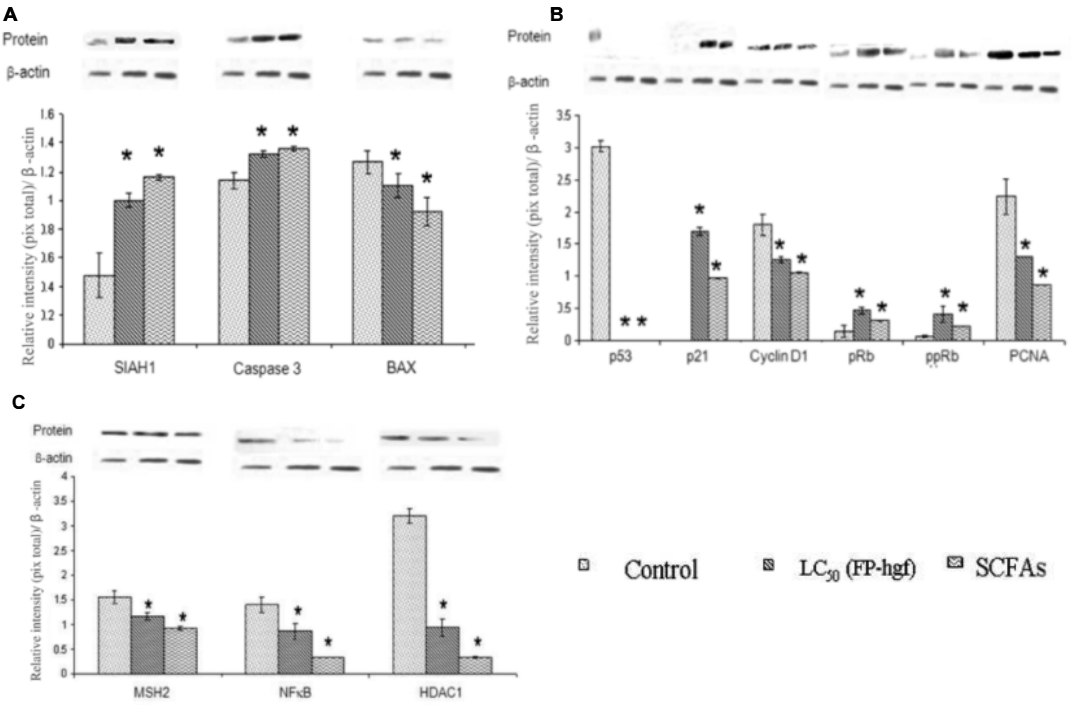

Figure 6. Expression of A) apoptosis-related proteins, B) cell cycle- related proteins and C) expression of MSH2, NFκB, and HDAC1 proteins-related proteins in HT-29 cells after 24 h of treatment with LC_{50}/FP-hgf and SCFAs mixture found in the LC_{50}/FP-hgf. Expression was analyzed by Western blot using specific antibodies. Control: protein expression in cells without any treatment. The blot was tested with anti-actin antibody to confirm equal protein loading. The protein expression was normalized to β-actin. Data are the mean ± standard errors of three independent experiments ($p < 0.05$ versus control). Adapted from Campos-Vega et al., (2012a).

Other mechanisms of action form beans have also been investigated. A Korean kidney bean husk extract exhibited a series of antitumor effects such as cell death and apoptotic body appearance. These antitumor potentials were accompanied by the increase in p-AMPK (AMP-activated protein kinase, possible target molecule of tumor control) and p-Acc as well as antitumor proteins p53 and p21 (Lee et al., 2009).

In the Four-Corners Breast Cancer Study, a relationship between bean consumption and reduced breast cancer risk was reported in which breast cancer incidence in Hispanic women who consumed a native Mexican diet (characterized by higher pulse consumption, such as common bean) was two-thirds that of the non-Hispanic white population whose diet was characterized as high in red meat, sugar and processed foods (Murtaugh et al., 2008). Common bean significantly

inhibits post-initiation stage of chemically induced mammary carcinogenesis in the rat (Thompson et al., 2009). The investigations of dietary bean consumption have centered on: (i) systemic factors (e.g. glucose-dependent growth factor signaling, inflammatory pathways), (ii) cell autonomous mechanisms (e.g. cellular energy and nutrient-sensing networks) such as the mammalian target of rapamycin (mTOR) network and (iii) signaling pathways through which systemic factors regulate cell proliferation and apoptosis. The emerging evidence shows that the mTOR network is deregulated in cardiovascular disease, type-2 diabetes, and in cancer, including breast cancer (Marshall, 2006; Um et al., 2006; Yang and Guan, 2007; Hynes and Boulay, 2006). Recently, cancer-associated molecular targets that mediate the effects of bean on cancer burden have been identified in a chemically induced rat model for breast cancer (Thompson et al., 2012). Beans reduce the carcinoma burden (62.2%, $P < 0.001$) with induction of apoptosis as the dominant cellular process. The observed changes are related with phosphorylation states of mammalian target of rapamycin (mTOR) substrates (4E-binding protein 1 and p70S6 kinase) and mTOR regulators adenosine monophosphate-activated protein kinase and protein kinase B (Akt) ($P < 0.001$); a reduced mTOR network activity in the liver is associated with an altered lipid metabolism (**Figure 7**). Identification of a role for the mTOR signaling network in the reduction of cancer burden by dietary bean is highly relevant given that this pathway is deregulated in the majority of human breast cancers.

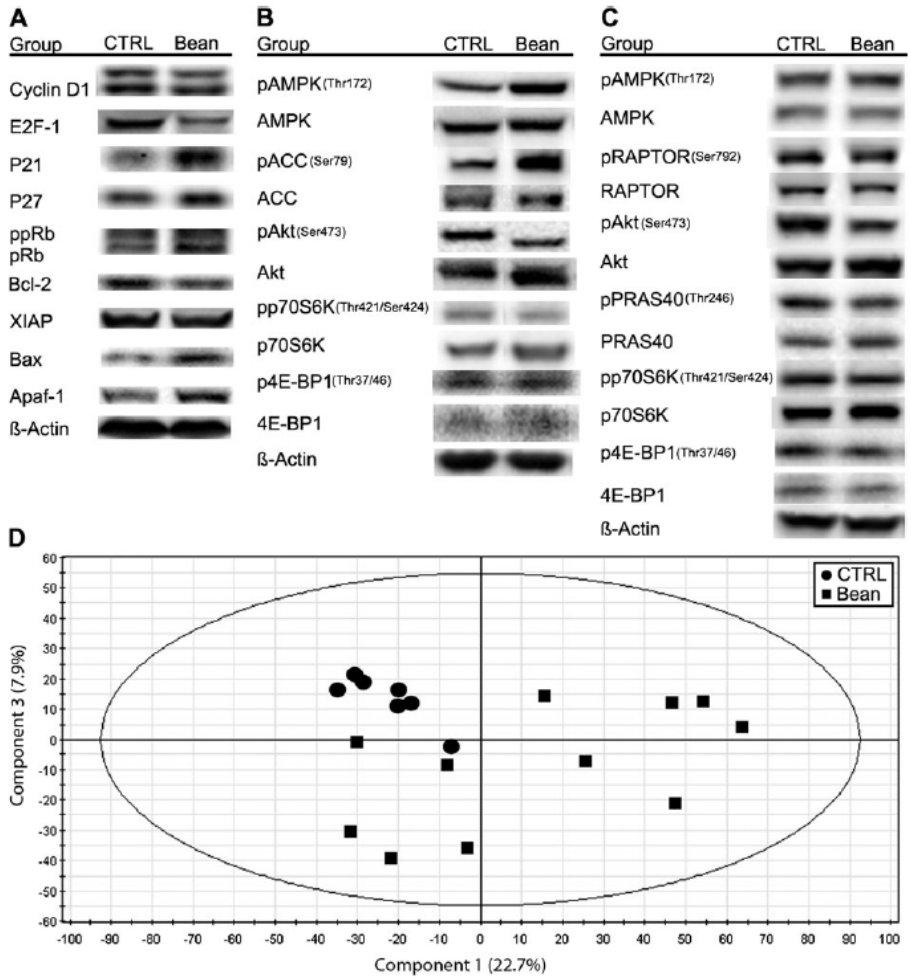

Figure 7. Effects of bean feeding on cell cycle and apoptosis regulators, mTOR signaling and the plasma metabolome. The images shown are those directly acquired from the ChemiDoc work station that is equipped with a CCD camera having a resolution of 1300 X 1030. (A) A composite image of representative western blots of lysates of carcinomas from control (CTRL) and bean-fed (bean) rats. Images are for cell cycle regulators, cyclin D1, E2F-1, p21Cip1, p27Kip1 and Rb (ppRb, hyper-phosphorylated Rb; pRb, hypo-phosphorylated Rb) and apoptosis regulators, Bcl-2, X-linked inhibitor of apoptosis protein (XIAP), Bax and Apaf-1. (B) A composite image of representative western blots of lysates of carcinomas from control (CTRL) and bean-fed (bean) rats. Images are for components of the AMPK-Akt-mTOR signaling network, phosphorylated and total: AMPK, ACC, Akt, p70S6 kinase (p70S6K) and 4E-binding protein 1 (4E-BP1). (C) A composite image of representative western blots of lysates of liver from control (CTRL) and bean-fed (bean) rats. Images are for phosphorylated and total: AMPK, Raptor, Akt, PRAS40, p70S6K and 4E-BP1. (D) Scores scatter plot from principal component analysis (PCA) of plasma demonstrating the separation between rats fed control diet (circles) and bean diet (60% wt/wt) (squares). (control, n 57; bean fed, n 51). From Thompson et al., (2012).

Besides, chromatography-time-of-flight MS has been undertaken to identify candidate metabolic processes that account for dry bean effects on disease risk with a specific focus on the development of breast cancer (Mensack et al., 2012). Principal component analysis (PCA) of mass spectral data reveals that tissue of both types from control-fed v. bean-fed rats could be distinguished by their metabolomic profiles. Candidate ion identification using MassTRIX analysis software reveals that alterations in eicosanoid, fatty acid, TAG and steroid metabolism partially accounted for the differences observed in both PCA. In addition, evidence was obtained consistent with the hypothesis that the varying inhibitory effects on mammary carcinogenesis of genetically distinct dry bean types were mirrored by differential patterns of lipid metabolites in mammary carcinoma. The use of MassTRIX provided links for metabolite profile enrichment with metabolic pathways in the Kyoto Encyclopedia of Genes and Genomes. Implicated pathways included a linkage between diacylglycerol and protein kinase C and eicosanoid metabolites and inducible cyclo-oxygenase-2 and/or eicosanoid degradation mediated via 15-PG dehydrogenase. These pathways have been reported to be misregulated during the development of cancer. The differences observed between control-fed and bean-fed rats in lipid metabolism require validation using targeted analytical methods and detailed analyses of how bean bioactive food components regulate genes that control lipid biosynthesis, interconversion and catabolism.

Although no evidence is available for the mechanism of action of common beans on other kind of cancer, the antiproliferative effect of legumes, including Adzuki beans, has been explored (Xu and Chang, 2012). Adzuki bean exhibited the strongest antiproliferative properties in a dose-dependent manner against all digestive system cancer cell lines (CAL27, AGS, HepG2, SW480 and Caco-2), ovary cancer cell SK-OV-3 and breast cancer cell MCF-7 among all legumes tested. A semi pure protein fraction, containing Bowman-Birk-type protease inhibitor from Tepary bean (*Phaseolus acutifolius*) seeds (TBPI), showed differential cytotoxic effect, as well as increase in cell attachment to culture dishes when tested for its *in vitro* effect on transformed cells, (García-Gasca et al., 2012). This TBPI was responsible for the increase in cell adhesion, decreasing culture dishes' extracellular matrix degradation, leading to reduced *in vitro* cell invasion capacity. This effect coincided with the suppression of Matrix Metalloproteinase-9 activity indicating that Tepary bean seeds contain at least 2 different groups of bioactive proteins with distinct effects on cancer cells. Nakaya et al., (2012) suggested that adzuki bean and its heat-stable extract are immunopotentiating foods that can be used as a dietary supplement for cancer prevention and immunotherapy. Adzuki bean (*Vigna angularis*) stimulates differentiation of bone marrow cells into immature dendritic cells with the greatest efficacy, when compared with 30 kinds of edible beans with biological activity. The level of IL-6 produced by sequential treatment of dendritic cells with Adzuki extract and lipopolysaccharide was the highest among the examined beans. Adzuki extract also inhibited the growth of human leukemia U937 cells, leading to induction of apoptosis.

Other compounds

Common beans are an excellent source of nutraceutical constituents such as fiber, protease inhibitors, phytic acid, and polyphenols such as tannins (Guzman-Maldonado et al., 1999). These compounds have antioxidant, antimutagenic, and anticarcinogenic activities and also free radical scavenging properties (de Mejía et al., 1999; Cardador-Martínez et al., 2002; Beninger and Hosfield et al., 2003). A recent report (Chan et al., 2013) showed the effects of quercetin, one of the main flavonoids found in beans, on the anti-tumor effect of trichostatin A (TSA), a novel anticancer drug, *in vitro* and *in vivo* and the possible mechanisms of these effects in human lung cancer cells. Quercetin significantly increases the growth arrest and apoptosis in A549 cells (expressing wild-type p53). However, such enhancing effects of quercetin are not significant in TSA-exposed H1299 cells (a p53 null mutant). Transfection of p53 siRNA into A549 cells significantly, but not completely diminishes the enhancing effects of quercetin on TSA-induced apoptosis. Furthermore, quercetin enhanced TSA-induced apoptosis through the mitochondrial pathway. Transfection of p53 siRNA abolished such enhancing effects of quercetin. However, quercetin increased the acetylation of histones H3 and H4 induced by TSA in A549 cells, even with p53 siRNA transfection as well as in H1299 cells (**Figure 8**). In a xenograft mouse model of lung cancer, quercetin enhanced the antitumor effect of TSA. Tumors from mice treated with TSA in combination with quercetin had higher p53 and apoptosis levels than those from control and TSA-treated mice. These data indicate that regulation of the expression of p53 by quercetin plays an important role in enhancing TSA-induced apoptosis in A549 cells. However, p53-independent mechanisms may also contribute to the enhancing effect of quercetin.

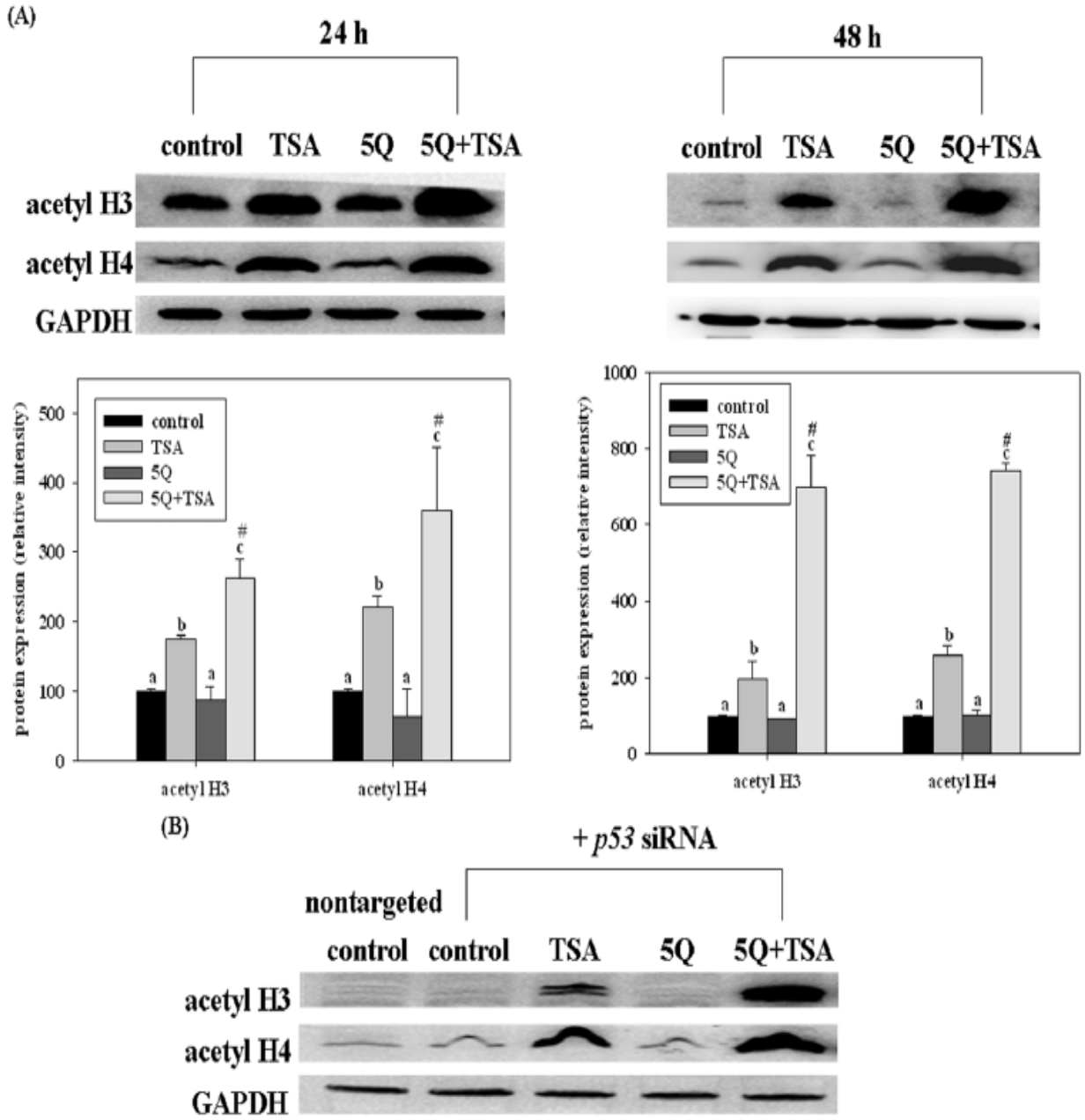

Figure 8. Effects of trichostatin A (TSA) alone or in combination with quercetin on the expression of acetyl histone H3 (acetyl H3) and H4 (acetyl H4) in A549 cells without (A) or with (B) p53 siRNA transfection. The cells were incubated with TSA (25 ng/mL) alone or in combination with 5 mM quercetin (5Q) for 24 or 48 h. Values (means 6 SD, n = 3) not sharing a common letter (a-c) are significantly different (p, 0.05). #denotes a significant interaction between TSA and quercetin (two-way ANOVA, p, 0.05). From Chang et al., (2013).

Choi and Kim (2013) investigated the antiproliferative activity of the isoflavones daidzein and genistein, present in common beans (Campos-Vega et al., 2009), in three breast cancer cell lines with different patterns of estrogen receptor (ER) and c erbB 2 protein expression (ERα-positive MCF 7 cells, c erbB 2 positive SK BR 3 cells, and ERα/c erbB 2 positive ZR 75 1). After treatment at various concentrations (1 200 µM for 72 h), the effect of daidzein and genistein on the proliferation of different cell types varied; these effects were associated with ERα and c erbB 2 expression. Ferulic acid, the most abundant phenolic acid in common beans (Campos-Vega et al., 2009), when combined with 2-deoxy-d-glucose (2DG) along with irradiation has been suggested as the mechanism of action on NCI-H460 (non-small cell lung carcinoma) cells alteration in expression of p53, p21, NF-κB, Bax, and caspase-3, indicating oxidative mechanism (Bandugula and N, 2013). Additionally, Prabhakar et al., (2012) explored the anti-cell proliferative efficacy of ferulic acid by analysing the expression pattern of cell proliferative markers, proliferating cellular nuclear antigen (PCNA) and cyclin D1, in the buccal mucosa of golden Syrian hamsters treated with 7, 12-dimethylbenz (a) anthracene (DMBA). Immunohistochemical (PCNA) and RT-PCR (Cyclin D1) analysis revealed over expression of PCNA and cyclin D1 (**Figure 9**) in the buccal mucosa of hamsters treated with DMBA alone (tumor bearing hamsters). Oral administration of ferulic acid at 40 mg/kg body weight to hamsters treated with DMBA not only completely prevented the tumor formation but also down regulated PCNA and cyclin D1 expression. The results suggest that ferulic acid may inhibit tumor formation in the buccal mucosa of hamsters treated with DMBA through its anti-cell proliferative potential as evidenced by decreased expression of PCNA and cyclin D1.

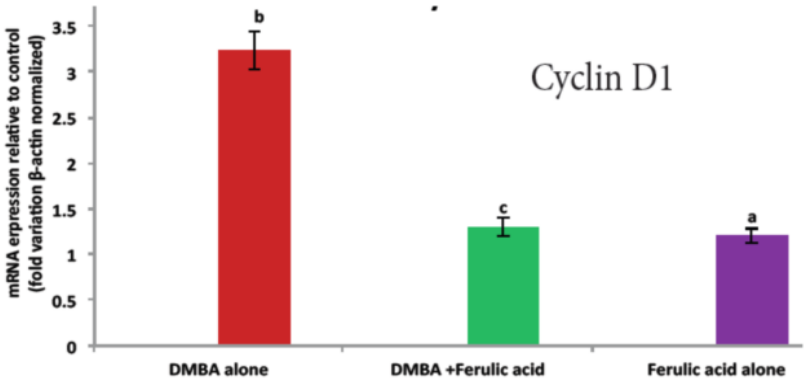

Figure 9. Pattern for Cyclin D1 in Hamsters Treated with DMBA Alone, DMBA+Ferulic Acid and Ferulic Acid alone. Values areexpressed as mean±SD for 10 hamsters in each group.* Values that do not share a common superscript letter between groups differ significantly at $p < 0.05$. (DMRT). From Prabhakar et al., (2012).

Anthocyanin-rich extracts from berries and grapes, and several pure anthocyanins and anthocyanidins exhibit pro-apoptotic effects in multiple cell types such as colon, breast, prostate, and leukemia cancer cells (Yuan et al., 2012). The main anthocyanins identified in seed coats of beans are delphinidin 3-glucoside 65.7%, petunidin 3-glucoside 24.3%, and maldivin 3-glucoside 8.7% (Campos-Vega et al., 2012b). Anthocyanins induce apoptosis through both intrinsic (mitochondrial) and extrinsic (Fas) pathways. In the intrinsic pathway, the treatment of cancer cells with anthocyanin results in destabilization of the mitochondrial membrane, cytochrome c release and activation of caspase-9, and -3 as well as pro-apoptotic protein such as apoptosis inducing factor. In the extrinsic pathway, anthocyanins modulate the expression of Fas and FasL (Fas ligand) in cancer cells, which result in the activation of caspase-8, then cleaves Bid to tBid, and ultimately stimulates cytochrome c release (Yuan et al., 2012). Furthermore, structure-activity studies suggest that the potency as inhibitors of epidermal growth factor receptor (EGFR), a target of an expanding class of anticancer therapies, may be positively correlated with the presence of hydroxyl functions in positions 3' and 5' of ring B of the anthocyanidin molecule, and inversely with the presence of methoxy groups in these positions. All of these findings provide important molecular basis for the antitumor properties of anthocyanidins (Yuan et al., 2012).

Riboflavin, a water-soluble vitamin that is a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) participates in various redox reactions, some of which are absolutely essential to the function of aerobic cells. Beans are sources of riboflavin (Campos-Vega et al., 2012b). Therefore, riboflavin deficiency in blood is expected to be related to a disturbance in riboflavin absorption. If inadequate intake of riboflavin exists, disturbances in the steps in intermediary metabolism may occur. Eli et al., (2012) identified down-regulated expression of riboflavin transporter 2 (RFT2) mRNA and protein as being closely related to the progression of gastric cancer (GC) lesions and also found a positive relationship between blood riboflavin levels and RFT2 protein expression as well as between blood riboflavin levels and development of GC. The RFT2 gene may be the key target of environmental and genetic factors in the development of GC. Zinc and iron are present in a variety of beans in high quantities and phytosterols in small quantities (Campos-Vega et al., 2012b). Zinc deficiency (ZD) increases the risk of esophageal squamous cell carcinoma (ESCC). In a rat model, chronic ZD induces an inflammatory gene signature that fuels ESCC development microRNAs, regulate gene expression and are aberrantly expressed in cancers. Alder et al., (2012) investigated chronic ZD (23 weeks) effect on protumorigenic microRNA signature induction and mapped microRNA profiles in ZD esophagus and six additional tissues (skin, lung, pancreas, liver, prostate and peripheral blood mononuclear cells using the nanoString technology [PBMC]). ZD overexpressed inflammation genes and altered microRNA expression (dysregulation of miR-31 and miR-21) across all tissues analyzed, predictive of disease development. Iron deficiency accelerates Helicobacter Pylori-induced carcinogenesis in rodents and humans (Noto et al., 2013). Several studies on cell cultures and xenograft mouse models suggest that dietary

phytosterols may also exert protective effects against common cancers. Llaverias et al., (2013) examined the effects of a dietary phytosterol supplement on tumor onset and progression using the well-characterized mammary tumor virus polyoma virus middle T antigen transgenic mouse model of inherited breast cancer. Both the development of mammary hyperplastic lesions (at age 4 weeks) and total tumor burden (at age 13 weeks) were reduced after dietary phytosterol supplementation in female mice fed a high-fat, high-cholesterol diet. A blind, detailed histopathologic examination of the mammary glands (at 8 weeks) also revealed the presence of less-advanced lesions in phytosterol-fed mice (**Figure 10**). This study provides preclinical proof of the concept that dietary phytosterols could prevent the tumor growth associated with fat-rich diet consumption.

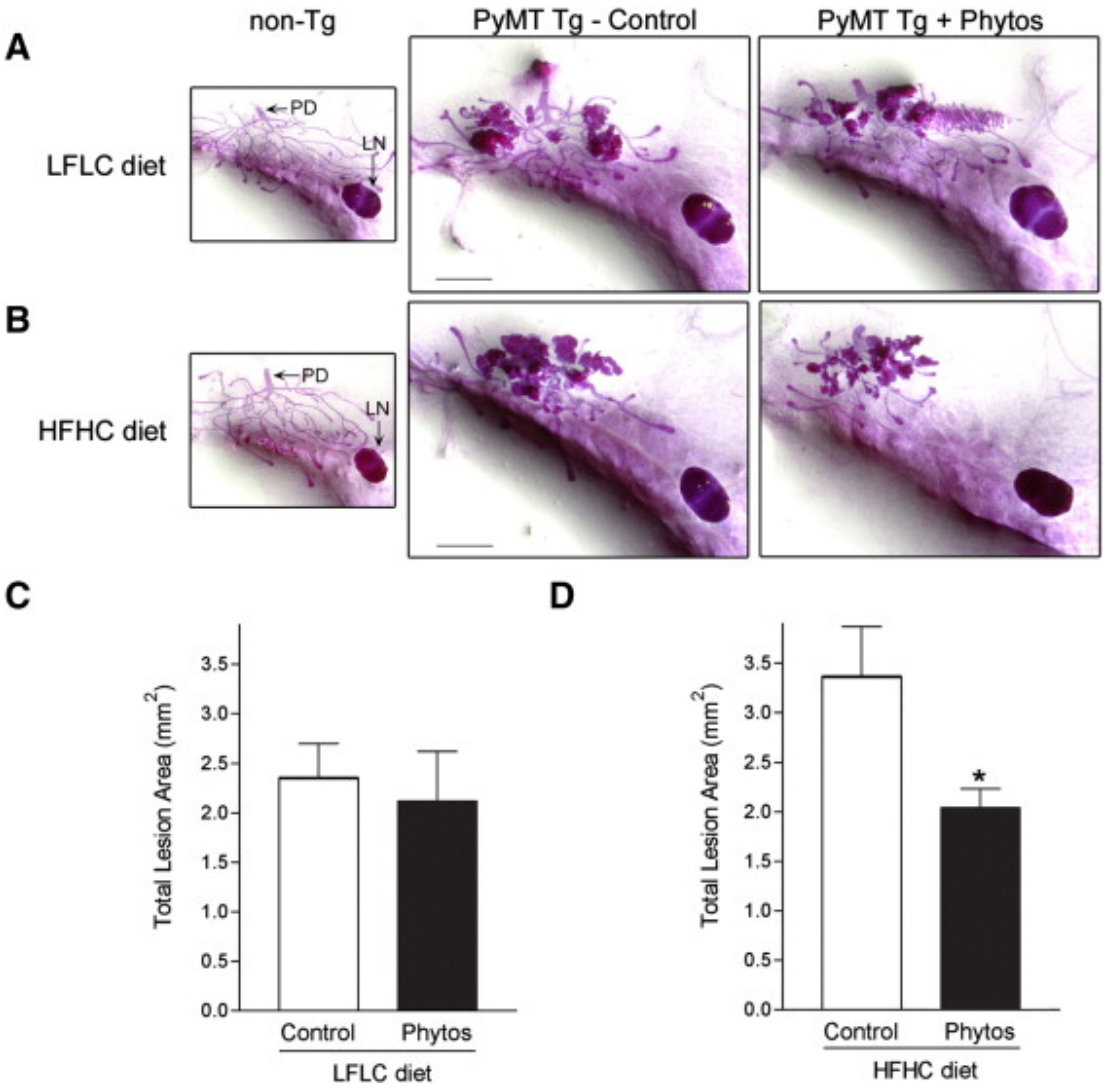

Figure 10. Phytosterol effect on the development of multifocal hyperplastic mammary lesions. Right inguinal mammary glands were harvested from 4-week-old PyMT Tg or non-Tg (control) female mice, fixed and stained with carmine dye. Representative, equally magnified images are shown for animals consuming and mice not consuming the phytosterol supplement in an LFLC diet (A) or an HFHC diet (B). The primary duct (PD) originates from the nipple area and is visible in the top left corner of all images. The subiliac lymph node (LN) is visible on the right of all images. Scale bars=1 mm. Quantification of the total area occupied by multifocal hyperplastic lesions in 4-week-old female PyMT Tg mice fed an LFLC diet (C) or (D) an HFHC diet. Data are expressed as mm2 of lesion per gland ±S.E.M. (n=8-11 in all experimental groups; P<.05). From Llaverias et al., (2013).

On the other hand, two key genes in the inflammatory process, cyclooxgenase-2 (COX-2) and nuclear factor kappaB (NF-kB), provide a mechanistic link between inflammation and cancer and are targets for chemoprevention and particularly, in colorectal cancer (Burstein and Fearon, 2008). In this regards, acetone extracts of mechanically obtained black bean hulls exhibit strong anti-inflammatory activity (Oomah et al., 2010) and used widely as prophylaxis may help protect against diseases associated with chronic inflammation by virtue of its aspirin-like COX inhibiting activity. This aspirin-like anti-inflammatory activity of hull extract may be responsible for the chemopreventive benefits of bean observed in cell and animal models and can be transferred to human studies since aspirin use is associated with 20-40% risk reduction in lung, stomach, breast and colon cancers (ecancer**,** 2011). This is confirmed by a recent patent (Gutierrez-Uribe et al., 2011) claiming that black bean phenolic antioxidants can treat, prevent and/or inhibit cancers or cancer cell (mammalian mammary, prostate, colon, hepatic, leukemia) growth, inhibit cholesterol synthesis (reducing cholesterol or LDL oxidation), reduce the symptom for calcium absorption in post-menopausal mammalian, and prevent chronic diseases such as cirrhosis. These beneficial effects results from the potential synergy among the phenolic compounds (anthocyanins, flavonoids, proanthocyanidins, flavones) and other phytochemicals (saponins, lectins, phytosterols) in black beans and/or hull extracts.

Concluding remarks

Dietary modification by increasing the consumption of a wide variety of common beans daily is a practical strategy for consumers to optimize their health and reduce the risk of cancer. Beans are good source of bioactive compounds and recent evidence provides information of their impact and mechanism of action on this pathology, mainly for non digestible fraction on colon cancer. Further research is warranted regarding the implications and the molecular ways in which common beans and their bioactive compounds modulate the development of different kinds of cancer.

REFERENCES

Alder H, Taccioli C, Chen H, Jiang Y, Smalley KJ, Fadda P, Ozer HG, Huebner K, Farber JL, Croce CM, Fong LY. Dysregulation of miR-31 and miR-21 induced by zinc deficiency promotes esophageal cancer. Carcinogenesis. 2012 Sep; 33(9):1736-44.

Armstrong B and Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer. 1975 Apr 15;15(4):617-31.

Bandugula VR and N RP. 2-Deoxy-D-glucose and ferulic acid modulates radiation response signaling in non-small cell lung cancer cells. Tumour Biol. 2013 Feb; 34(1):251-9.

Bazzano LA, He J, Ogden LG, Loria C, Vupputuri S, Myers L, Whelton PK. Legume consumption and risk of coronary heart disease in US men and women: NHANES I Epidemiologic Follow-up Study. Arch Intern Med. 2001 Nov 26; 161(21):2573-8.

Beninger CW and Hosfield GL. Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from Phaseolus Vulgaris L. seed coat color genotypes. J Agric Food Chem. 2003 Dec 31; 51(27):7879-83.

Bennink MR and Om AS. Inhibition of Colon Cancer (CC) by soy phytochemicals but not by soy protein. FASEB J. 1998; 12(5):A655.

Bennink MR, Om AS, Miyagi Y. Inhibition of colon cancer (CC) by soy flour but not by genistin or a mixture of isoflavones. FASEB J. 1999; 13:A50.

Bobe G, Barrett KG, Mentor-Marcel RA, Saffiotti U, Young MR, Colburn NH, Albert PS, Bennink MR, Lanza E. Dietary cooked navy beans and their fractions attenuate colon carcinogenesis in azoxymethane-induced ob/ob mice. Nutr Cancer. 2008; 60(3):373-81.

Burstein E, Fearon ER. Colitis and cancer: a tale of inflammatory cells and their cytokines. J Clin Invest 2008, 118:464-467.

Campos-Vega R, García-Gasca T, Guevara-González R, Ramos-Gómez M, Oomah BD, Loarca-Piña G. Human gut flora-fermented nondigestible fraction from cooked bean (*Phaseolus vulgaris* L.) modifies protein expression associated with apoptosis, cell cycle arrest, and proliferation in human adenocarcinoma colon cancer cells. J Agric Food Chem. 2012a Dec 26; 60(51):12443-50.

Campos-Vega R, Guevara-Gonzalez R, Guevara-Olvera B, Oomah BD, Loarca-Piña G. Bean (*Phaseolus vulgaris* L.) polysaccharides modulate gene expression in human colon cancer cells (HT-29). Food Res. 2010b Jan; 43(4): 1057 - 1064.

Campos-Vega R, Loarca-Piña G, Oomah BD. Minor components of pulses and their potential impact on human health. Food Res. 2010a Mar; (2):461-82.

Campos-Vega R, Reynoso-Camacho R, Pedraza-Aboytes G, Acosta-Gallegos JA, Guzman-Maldonado SH, Paredes-Lopez O, Oomah BD, Loarca-Piña GF. Chemical composition and in vitro polysaccharide fermentation of different beans (*Phaseolus vulgaris* L.). J Food Sci. 2009; 74(7):T59-65.

Campos-Vega R, Vergara-Castañeda HA, Oomah BD. Functional compounds sources: Beans in sight. Bioactive Compounds: Types, Biological Activities; Popescu E, Golubev I, Ed: Nova Science Publishers, Inc. 2012b.

Campos-Vega R, Vergara-Castañeda HA, Oomah BD. Functional Food Sources: Beans in Sight. Pages 1-56 *in* Popescu, E., and Golubev, I. (Eds.)., Beans: Nutrition, Consumption and Health, Nova Science Publishers, New York, 2011.

Cardador-Martínez A, Loarca-Piña G, Oomah BD. Antioxidant activity in common beans (*Phaseolus vulgaris* L.). J Agric Food Chem. 2002 Nov 20; 50(24):6975-80.

Chan ST, Yang NC, Huang CS, Liao JW, Yeh SL. Quercetin enhances the antitumor activity of trichostatin A through upregulation of p53 protein expression in vitro and in vivo. PLoS One. 2013; 8(1):e54255.

Choi EJ and Kim GH. Antiproliferative activity of daidzein and genistein may be related to ERα/c-erbB-2 expression in human breast cancer cells. Mol Med Report. 2013 Jan 21.

Correa P. Epidemiological correlations between diet and cancer frequency. Cancer Res. 1981 Sep; 41(9 Pt 2):3685-90.

Cruz-Bravo RK, Guevara-González R, Ramos-Gómez M, García-Gasca T, Campos-Vega R, Oomah BD, Loarca-Piña G. Fermented nondigestible fraction from common bean (*Phaseolus vulgaris* L.) cultivar Negro 8025 modulates HT-29 cell behavior. J Food Sci. 2011 Mar; 76(2):T41-7.

Dahm CC, Keogh RH, Spencer EA, Greenwood DC, Key TJ, Fentiman IS, Shipley MJ, Brunner EJ, Cade JE, Burley VJ, Mishra G, Stephen AM, Kuh D, White IR, Luben R, Lentjes MA, Khaw KT, Rodwell Bingham SA. Dietary fiber and colorectal cancer risk: a nested case-control study using food diaries. J Natl Cancer Inst. 2010 May 5; 102(9):614-26.

de Mejía EG, Castaño-Tostado E, Loarca-Piña G. Antimutagenic effects of natural phenolic compounds in beans. Mutat Res. 1999 Apr 26; 441(1):1-9.

Delzenne N, Cherbut C, Neyrinck A. Prebiotics: actual and potential effects in inflammatory and malignant colonic diseases. Curr Opin Clin Nutr Metab Care. 2003 Sep; 6(5):581-6.

Doll R and Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst. 1981 Jun; 66(6):1191-308.

ecancer (2011). Aspirin, salicylates and cancer: report of a meeting at the Royal Society of Medicine, London, 23 November 2010. DOI: 10:3332/ecancer.2011.213, published 24/05/2011. http://ecancer.org/ecms/5/213/pdf.

Eli M, Li DS, Zhang WW, Kong B, Du CS, Wumar M, Mamtimin B, Sheyhidin I, Hasim A. Decreased blood riboflavin levels are correlated with defective expression of RFT2 gene in gastric cancer. World J Gastroenterol. 2012 Jun 28; 18(24):3112-8.

Feregrino-Pérez AA, Berumen LC, García-Alcocer G, Guevara-González RG, Ramos-Gómez M, Reynoso-Camacho R, Acosta-Gallegos JA, Loarca-Piña G. Composition and chemopreventive effect of polysaccharides from common beans (*Phaseolus vulgaris* L.) on azoxymethane-induced colon cancer. J Agric Food Chem. 2008 Sep 24; 56(18):8737-44.

Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Cancer incidence and mortality Worldwide. IARC Cancer Base, n.º 10; Centro Internacional de Investigaciones sobre el Cáncer; GLOBOCAN 2008; Lyon, Francia. 2010.

García-Gasca T, García-Cruz M, Hernández-Rivera E, López-Matínez J, Castañeda-Cuevas AL, Yllescas-Gasca L, Rodríguez-Méndez AJ, Mendiola-Olaya E, Castro-Guillén JL, Blanco-Labra A. Effects of tepary bean (*Phaseolus acutifolius*) protease inhibitor and semi pure lectin fractions on cancer cells. Nutr Cancer. 2012; 64(8):1269-78.

Guzmán-Maldonado SH, Paredes-López O. Biotechnology for the improvement of nutritional quality of food crop plants. Molecular Biotechnology for Plant Food Production; Paredes-Lopez O, Ed; Technomic Publishing: Lancaster PA. 1999:553-620.

Hangen L and Bennink MR. Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. Nutr Cancer. 2002; 44(1):60-5.

Hynes NE and Boulay A. The mTOR pathway in breast cancer. J Mammary Gland Biol Neoplasia. 2006 Jan; 11(1):53-61.

Kolonel LN, Hankin JH, Whittemore AS, Wu AH, Gallagher RP, Wilkens LR, John EM, Howe GR, Dreon DM, West DW, Paffenbarger RS Jr. Vegetables, fruits, legumes and prostate cancer: a multiethnic case-control study. Cancer Epidemiol Biomarkers Prev. 2000 Aug; 9(8):795-804.

Lanza E, Hartman TJ, Albert PS, Shields R, Slattery M, Caan B, Paskett E, Iber F, Kikendall JW, Lance P, Daston C, Schatzkin A. High dry bean intake and reduced risk of advanced colorectal adenoma recurrence among participants in the polyp prevention trial. J Nutr. 2006 Jul; 136(7):1896-903.

Lee YK, Hwang JT, Lee MS, Kim YM, Park OJ. Kidney bean husk extracts exert antitumor effect by inducing apoptosis involving AMP-activated protein kinase signaling pathway. Ann N Y Acad Sci. 2009 Aug; 1171:484-8.

Llaverias G, Escolà-Gil JC, Lerma E, Julve J, Pons C, Cabré A, Cofán M, Ros E, Sánchez-Quesada JL, Blanco-Vaca F. Phytosterols inhibit the tumor growth and lipoprotein oxidizability induced by a high-fat diet in mice with inherited breast cancer. J Nutr Biochem. 2013 Jan; 24(1):39-48.

Marshall S. Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity, and cancer. Sci STKE. 2006 Aug 1; 2006(346):re7.

Mensack MM, McGinley JN, Thompson HJ. Metabolomic analysis of the effects of edible dry beans (Phaseolus vulgaris L.) on tissue lipid metabolism and carcinogenesis in rats. Br J Nutr. 2012 Aug; 108 Suppl 1:S155-65.

Mentor-Marcel RA, Bobe G, Barrett KG, Young MR, Albert PS, Bennink MR, Lanza E, Colburn NH. Inflammation-associated serum and colon markers as indicators of dietary attenuation of colon carcinogenesis in ob/ob mice. Cancer Prev Res (Phila). 2009 Jan; 2(1):60-9.

Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer. 1989 Aug 1; 64(3):598-604.

Murtaugh MA, Sweeney C, Giuliano AR, Herrick JS, Hines L, Byers T, Baumgartner KB, Slattery ML. Diet patterns and breast cancer risk in Hispanic and non-Hispanic white women: the Four-Corners Breast Cancer Study. Am J Clin Nutr. 2008 Apr; 87(4):978-84.

Nakaya K, Nabata Y, Ichiyanagi T, An WW. Stimulation of dendritic cell maturation and induction of apoptosis in leukemia cells by a heat-stable extract from azuki bean (*Vigna angularis*), a promising immunopotentiating food and dietary supplement for cancer prevention. Asian Pac J Cancer Prev. 2012; 13(2):607-11.

Noto JM, Gaddy JA, Lee JY, Piazuelo MB, Friedman DB, Colvin DC, Romero-Gallo J, Suarez G, Loh J, Slaughter JC, Tan S, Morgan DR, Wilson KT, Bravo LE, Correa P, Cover TL, Amieva MR, Peek RM Jr. Iron deficiency accelerates Helicobacter pylori-induced carcinogenesis in rodents and humans. J Clin Invest. 2013 Jan 2; 123(1):479-92.

Oomah BD, Corbé A, Balasubramanian P. Antioxidant and anti-inflammatory activities of bean (*Phaseolus vulgaris* L.) hulls. J Agric Food Chem 2010, 58, 8225-8230.

Prabhakar MM, Vasudevan K, Karthikeyan S, Baskaran N, Silvan S, Manoharan S. Anti-cell proliferative efficacy of ferulic acid against 7, 12-dimethylbenz(a) anthracene induced hamster buccal pouch carcinogenesis. Asian Pac J Cancer Prev. 2012; 13(10):5207-11.

Rondini EA and Bennink MR. Microarray Analyses of Genes Differentially Expressed by Diet (Black Beans and Soy Flour) during Azoxymethane-Induced Colon Carcinogenesis in Rats. J Nutr Metab. 2012; 351796.

Sengupta S, Muir JG, Gibson PR. Does butyrate protect from colorectal cancer? J Gastroenterol Hepatol. 2006 Jan; 21:209-18.

Thompson MD, Brick MA, McGinley JN, Thompson HJ. Chemical Composition and Mammary Cancer Inhibitory Activity of Dry Bean. Crop Sci. 2009; 49(1):179-186.

Thompson MD, Mensack MM, Jiang W, Zhu Z, Lewis MR, McGinley JN, Brick MA, Thompson HJ. Cell signaling pathways associated with a reduction in mammary cancer burden by dietary common bean (Phaseolus vulgaris L.). Carcinogenesis. 2012 Jan; 33(1):226-32.

Um SH, D'Alessio D, Thomas G. Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1. Cell Metab. 2006 Jun; 3(6):393-402.

Uribe J, Serna-Saldivar S, Moreno-Cuevas J, Hernandes-Brenes C, Guajardo-Touche E. Cancer cell growth inhibition by black bean (*Phaseolus vulgaris* L) extracts. European Patent Application, EP 2311476 A1, published 20.04.2011.

Vergara-Castañeda HA, Guevara-González RG, Guevara-Olvera BL, Oomah, B.D., et Loarca-Piña, G.F. Non-digestible fraction of beans (*Phaseolus vulgaris* L.) modulates signalling pathway genes at an early stage of colon cancer in Sprague-Dawley rats. Br J Nutr. 2012; 108:S145-54.

Vergara-Castañeda HA, Guevara-González RG, Ramos-Gómez M, Reynoso-Camacho R, Guzmán-Maldonado H, Feregrino-Pérez AA, Oomah BD, Loarca-Piña G. Non-digestible fraction of cooked bean (*Phaseolus vulgaris* L.) cultivar Bayo Madero suppresses colonic aberrant crypt foci in azoxymethane-induced rats Food Funct. 2010 Dec; 1(3):294-300.

Waldecker M, Kautenburger T, Daumann H, Busch C, Schrenk D. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. J Nutr Biochem. 2008 Sep; 19(9):587-93.

WCRF and AICR. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. World Cancer Research Fund and American Institute for Cancer Research; Washington DC. 2007.

Xu B and Chang SKC. Comparative study on antiproliferation properties and cellular antioxidant activities of commonly consumed food legumes against nine human cancer cell lines. Food Chem. 2012 Oct; 134(3):1287-96.

Yang Q and Guan KL. Expanding mTOR signaling. Cell Res. 2007 Aug; 17(8):666-81.

Yuan B, Imai M, Kikuchi H, Fukushima S, Hazama S, Akaike T, Yoshino Y, Ohyama K, Hu X, Pei X, Toyoda H. Cytocidal effects of polyphenolic compounds, alone or in combination with, anticancer drugs against cancer cells: potential future application of the combinatory therapy. Apoptosis and medicine; Ntuli TB, Ed; INTECH. 2012.