Chapter 3

Modulation of Apoptosis in Colon Cancer Cells by Bioactive Compounds

Marinela Bostan, Mirela Mihaila, Camelia Hotnog, Coralia Bleotu, Gabriela Anton, Viviana Roman and Lorelei Irina Brasoveanu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/63382

Abstract

A big challenge for a successful colon cancer treatment is the lack of eradication of the entire tumour cell population and consequent development of chemoresistance. Control of cell number from tissues and elimination of cells predisposed to malignant transformation, having an aberrant cell cycle or presenting DNA mutations, might be performed by a cellular 'suicide' mechanism — the programmed cell death, or apoptosis. Coordinated activation and execution of multiple subprograms are needed, added by a good knowledge of the basic components of the death machinery, besides their interaction to regulate apoptosis in a coordinated manner. Triggering apoptosis in target cells is a key mechanism by which chemotherapy promotes cell killing. Many anti-cancer drugs act during physiological pathways of apoptosis, leading to tumour cell destruction. New therapeutic approaches in cancer induce tumour cells to undergo apoptosis and break the cancer cell resistance to apoptosis commands. Administrations of natural compounds that prevent induction, inhibit or delay the progression of cancer, or induce inhibition or reversal of carcinogenesis at a premalignant stage represent chemoprevention strategies. Several natural compounds have been shown to be promising based on their anti-cancer effects and low toxicity; alternative approaches might be taken into account to obtain a stronger anti-tumour response when lower concentrations of anti-cancer drugs are used, and to diminish the undesirable side-effects.

Keywords: colon cancer, apoptosis, tumour evasion, bioactive compounds, combined therapy

1. Introduction

Cancer is a disease of cells that is thought to evolve along a multi-step process: the transformation of normal cells, tumour progression and advanced metastasis, that involve a complex series of events such as genetic alterations, aberrant progression of the cell cycle, resistance to growth inhibition, proliferation without dependence on growth factors, replication without limit, evasion of apoptosis, induction of angiogenesis and modification of cell adhesion [1]. For an accurate prediction, prevention, early detection and development of anti-cancer drugs, it is essential to identify the stages of development and use basic information [2]. The lack of eradication of the entire tumour cell population and the consequent development of chemoresistance represent main obstacles to a successful treatment in many malignancies, including colon cancer [3, 4]. The control of cell number from tissues and elimination of those predisposed to malignant transformation, having an aberrant cell cycle or presenting DNA mutations, might be performed by a cellular "suicide" mechanism, the programmed cell death or apoptosis [5, 6]. Elucidating the mechanisms of programmed cell death process seems to be of great importance for carcinogenesis, tumour evasion, and to have practical implications for anti-cancer therapy since many anti-cancer drugs act during physiological pathways of apoptosis, leading to tumour cell destruction [7, 8].

Several therapeutic agents used in colon cancer treatment, e.g. fluoropyrimidines, cisplatin, oxaliplatin, irinotecan have been shown to induce resistance in cancer cell killing, and their number are rapidly increasing, possibly through the modulation of survival cell components, such as proliferative or anti-apoptotic proteins [9, 10]. Triggering apoptosis in target cells represents a key mechanism by which chemotherapy promotes cell killing. Continuing efforts are made for discovering new molecular target-based molecules [11], and new therapeutic approaches in colon cancer involve restored cellular mechanisms responsible for the induction of apoptosis in tumour cells [12–15].

A main strategy in colon cancer treatment might be the combined multi-drug chemotherapy, the reason being the potential additive or synergistic tumour cytotoxicity produced [1]. The focus on finding new therapeutic strategies has recently shifted to natural products. Various plants and their bioactive compounds have been shown to have anti-carcinogenic and anti-proliferative effects towards the colon cancer cells. Studies have also reported positive correlation between the antioxidant activity of plants and their anti-proliferative effects, suggesting the potential action of antioxidants in inhibiting cancer cell growth. For example, the flavonoids display a wide range of biological activities, including anti-inflammatory and cytoprotective activities, and several are known to act as anti-cancer reagents [16].

The administration of synthetic or natural compounds that prevent induction of cancer, inhibit or delay its progression, or reverse carcinogenesis at a premalignant stage could represent useful strategies because of their potential clinical application in combined treatments with anti-cancer drugs [17]. By combining natural compounds with anti-cancer drugs, it might be obtained an increase of cancer treatment effects, specifically in highly invasive colon cancer cells, while in non-tumour cells the use of natural compounds could reduce the cytotoxic side effects [18].

http://dx.doi.org/10.5772/63382

2. Biology of colon cells: normal versus carcinogenic

Colorectal cancer (CRC) is the third most common malignancy worldwide, being frequently diagnosed in advanced stages. Recent data added to the molecular explanations of growth dysregulation, metastasis formation, extension of life span, and loss of maintenance of genomic and epigenetic integrity in cancer suggest models for their causal connection. The mechanisms of growth control, senescence, and anchorage dependence are linked at the molecular level [2].

The adult colon epithelium contains three cell types that arise from a multipotent stem cell: absorptive epithelial, enteroendocrine and Goblet cells. Colonic epithelial cells are configured in deep invaginations into the wall of the colon named crypts: from stem cells located at the base of the crypt, they arise and migrate to the luminal surface of the crypt where they are shed. Stem cells divide asymmetrically: the "old" DNA is retained in the stem cell population, and the new synthesised DNA is donated to daughter cells that migrate up the crypt and are ultimately shed. Stem cells are particularly vulnerable to developing mutations that might evolve into a malignant clone. Therefore, the cells located at the base of crypts, presumably stem cells, are highly prone to apoptosis, able to counteract dangerous mutations [19]. The result of the imbalance between cell proliferation and apoptosis determines colorectal tumour growth. Relatively undifferentiated tumours with higher proliferative potential are often more aggressive than well-differentiated ones [2]. The molecular mechanisms of cell division and apoptosis are similar in normal and tumour cells, but in tumour cells, these mechanisms are aberrantly regulated. Four cellular functions are inadequate regulated in tumour cells: (1) control of cell proliferation is inefficient; (2) genetic and chromosomal structure is destabilized; (3) cellular differentiation program is frequently altered; (4) the control of apoptosis is disturbed [20].

Multiple sequential genetic changes are needed to occur in order to ensure colorectal cancer evolution. During progression of normal epithelial to carcinoma cell in colorectal cancer, TP53, KRAS, BRAF and PIK3CA gene alterations play important roles. Gene alterations cause disruption of signalling pathways in which they are involved, accompanied by increased proliferative potential and decreased apoptosis of cells [21]. Along with genetic mutations, colon carcinogenesis is accompanied by epigenetic changes that lead to altered expression of key genes. Three major epigenetic regulatory mechanisms are described: (a) DNA methylation, (b) the covalent modifications of histones and (c) non-coding RNA interference [22].

3. Programmed cell death in normal versus carcinogenic colon cells

Apoptosis represents a cellular "suicide" mechanism which allows control of the number of cells from tissues and removal of cells that present DNA mutations or have an aberrant cell cycle, predisposed to malignant transformation [5]. Thus, elucidating the mechanisms of programmed cell death process seems to be of great importance for malignant transformation, tumour evasion, and therefore for anti-cancer therapy like restoration of cellular mechanisms responsible for the induction of apoptosis in tumour cells [23, 24]. Abnormalities in apoptotic

function contribute to both pathogenesis of colorectal cancer, and its resistance to chemotherapeutics and radiotherapy [19].

3.1. Apoptosis pathways

Apoptosis is an active, specialized form of cell death with distinct biochemical and genetic pathways that play a critical role in normal tissue homeostasis and development. Under stress, such as precancerous lesions, the mechanisms involved in repairing DNA damage are activated and potentially harmful cells are removed, and carcinogenesis is blocked [25]. Lack of regulation of the apoptosis pathways may promote tumorigenesis and induce resistance to treatment in cancer cells [19].

The apoptotic process displays morphological features of the cells: cellular shrinkage with nuclear chromatin condensation and nuclear fragmentation, membrane blebbing, and cell-self-fragmentation into apoptotic bodies. Apoptosis is initiated by two basic signalling pathways: **the extrinsic pathway**, initiated by external stimuli and via activation of death receptors on the cell surface, such as tumour necrosis factor-α (TNF-α), Fas (CD95/APO1) and TNF-related apoptosis-inducing ligand (TRAIL) receptors, and **the intrinsic** (or mitochondrial) **pathway,** activated by intracellular stimuli and characterized by mitochondrial outer membrane permeabilization and release of mitochondrial cytochrome *c* (cyt-c) [26]. There is an overlap between the two apoptotic pathways: the extrinsic pathway usually also activates the intrinsic pathway, and both pathways result in the recruitment and activation of cysteine-aspartic acid proteases (caspases) [27, 28]. Upon receiving specific signals instructing the cells to undergo apoptosis, the caspase family of proteins is typically activated and cleaves key cellular components required for normal cellular function, including structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes [29]. Caspases can directly signal apoptosis or use mitochondria as an intermediate and additional point of regulation in apoptosis signalling [30].

(a) the mitochondrial pathway (**the intrinsic pathway**) is activated by a wide variety of cytotoxic drugs, DNA damage, growth factor deprivation, oxidative stress, Ca^{2+} overload and oncogene activation [29, 30]. It is regulated by formation of the mitochondrial permeability transition pore (MPTP), composed by Bcl-2 family members and voltage-dependent anion channels on the outer mitochondrial membrane [31], leading to mitochondrial outer membrane permeabilization (MOMP). The drop of mitochondrial membrane potential initiates the osmotic swelling of the matrix by water influx and release of cytochrome *c* from mitochondrial intermembrane space into the cytoplasm. Cytochrome *c* then associates with apoptotic protease-activating factor 1 (APAF-1) and caspase-9 forming apoptosome complex. The activation of caspase-9 and/or caspase-8 leads to caspase-3 cleavage, endonuclease activation, and ultimately nuclear DNA fragmentation, which is the hallmark of apoptosis [31, 32]. B cell leukaemia/lymphoma 2 (Bcl-2) family proteins are central regulators of the intrinsic pathway, which either suppress or promote changes in mitochondrial membrane permeability required for the release of cyt-c and other apoptogenic proteins [33].

(b) the extrinsic pathway starts with the stimulation of specific death receptors upon binding of their ligands, like tumour necrosis factor (TNF), tumour necrosis factor-related apoptosis-

inducing ligand (TRAIL) and CD95 (Fas or APO1) [34]. Death receptors are transmembrane proteins with a death domain in their cytosolic region; the ligands binding causes oligomerization of these receptors, exposing their death domains (DD) in their cytosolic tail, which rapidly bind to Fas-Associated Death Domain (FADD). Several of the DD-containing TNF-family receptors use caspase activation as a signalling mechanism, including TNFR1/CD120a, Fas/APO1/CD95, DR3/Apo2/Weasle, DR4/TrailR1, DR5/TrailR2, and DR6 [35, 36]. Binding of these receptors at the cell surface results in the recruitment of several intracellular proteins, including some procaspases, to the cytosolic domains of these receptors, forming a "death-inducing signalling complex" (DISC) that triggers caspase-8 activation [37, 38]. In the case of TNFR1, after the ligand binds to TNFR1, the cytosolic region of the receptor does not bind FADD, but TRADD adaptor, as well as several other signalling proteins, some of them being involved in the activation of NF-κB transcription factor. The initial complex is then released from the receptor, TRADD binds to FADD in the cytosol, and caspase-8 is recruited. The downstream signalling depends on additional interactions with proteins like FLICE-like inhibitory proteins (c-FLIP), forming a complex that contains heterodimers of caspase-8 and c-FLIP that will inhibit apoptosis. However, if NF-κB activity is blocked or disrupted, or c-FLIP expression is inhibited, caspase-8 is activated and cell undergoes apoptosis [30, 39]. Between the death receptor pathway and the mitochondrial pathway of apoptosis, there is an overlap. Caspase-8 has another substrate in the cell, BID, a BH3-only protein: when caspase-8 cleaves BID, the protein is translocated to the mitochondria to promote MOMP and to initiate mitochondria-dependent apoptosis [40].

3.2. Evasion mechanisms of apoptosis in colon cancer

Apoptosis is subverted during tumorigenesis through the systematic loss of regulatory control mechanisms, ultimately resulting in the generation of a malignant phenotype and resistance to chemotherapy and radiation therapy. Several potential mechanisms and factors involved were taken into account to explain the defects in apoptotic signalling and the increased activation of anti-apoptotic pathways that were observed in colon cancer cells:

(a) Disrupted balance between pro- and anti-apoptotic proteins

Many proteins exert pro- or anti-apoptotic activity in cells, and the ratio between them plays an important role in the regulation of cell death. Over- or under-expressed genes were also found to contribute to carcinogenesis by reducing apoptosis in cancer cells. Key regulatory proteins of apoptotic machinery, such as Bcl-2 (including Bcl-xl and Bax) and IAP family, undergo changes in expression during the transition from adenoma to carcinoma, and therefore, they were used as prognostic biomarkers [7]. Pro- and anti-apoptotic mediators can regulate mitochondrial outer membrane permeability and release of cytochrome *c* from the mitochondria into the cytoplasm [41] (**Figure 1**).

When there is a disruption in the balance of anti-apoptotic and pro-apoptotic members of the **Bcl-2 family**, the result is a dysregulation of apoptosis in the affected cells. This can be due to the overexpression of one or more anti-apoptotic proteins, or the underexpression of one or more pro-apoptotic proteins, or both [42, 43]. In colorectal cancer, the dysregulated expression

of Bcl-2 family members may be associated with disease outcomes. Bcl-2 expression is restricted to the basal epithelial cells in normal and hyperplastic mucosa, but in dysplastic polyps and carcinomas, it is extended to the parabasal and superficial regions. Bcl-2 expression is increased in hyperplastic polyps and markedly increased in almost all adenomas, while carcinomas show weaker Bcl-2 expression, indicating the decrease of apoptosis during progression from adenoma to carcinoma [44, 45].

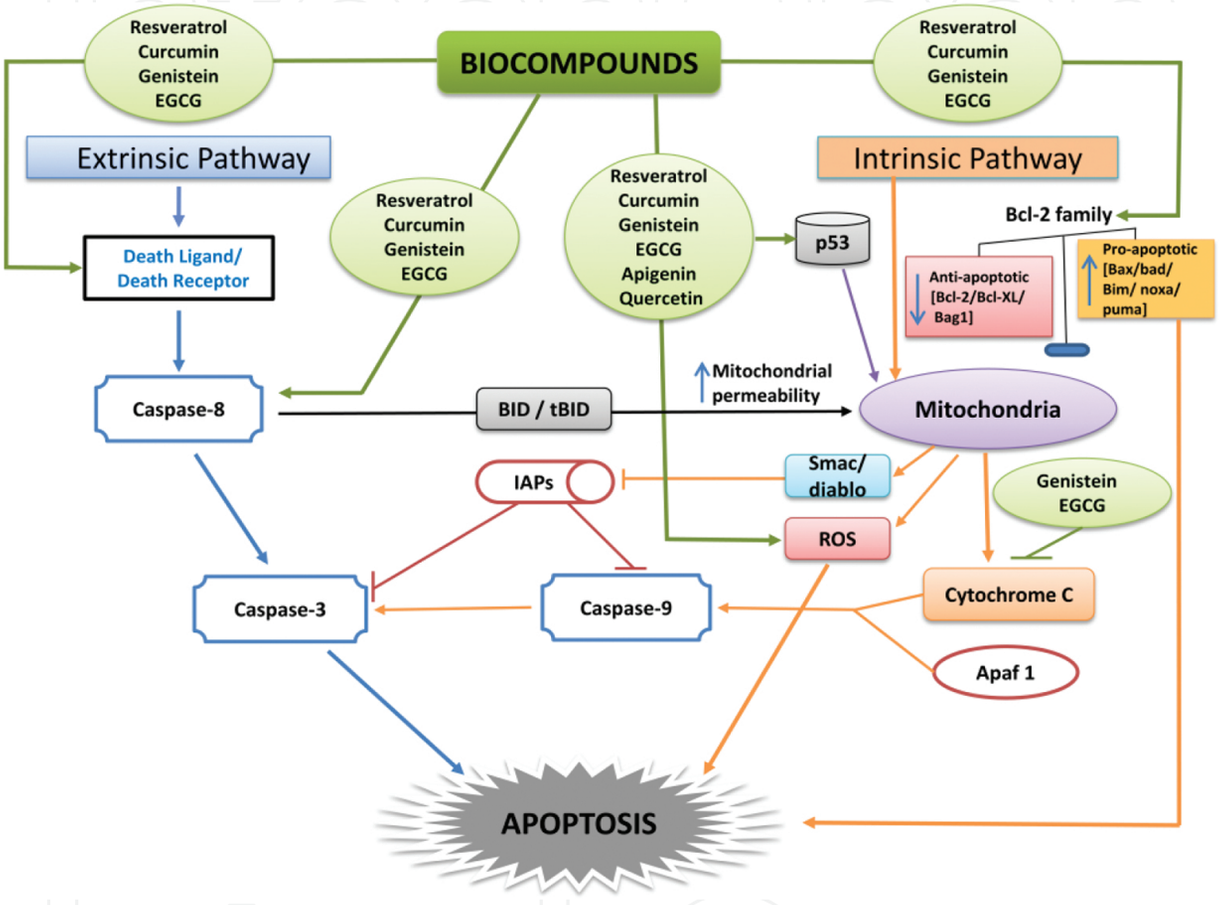

Figure 1. Role of biocompounds in modulation of intrinsic and extrinsic pathways of apoptosis.

Overexpression of the anti-apoptotic Bcl-2 family member Bcl-XL predicts poor prognosis in patients with colonic adenocarcinomas, conferring a multidrug resistance phenotype [46, 47]. Bcl-w, another anti-apoptotic Bcl-2 family protein, plays a general role in the progression from adenoma to adenocarcinoma in the colorectal epithelium; it is frequently expressed in colorectal adenocarcinomas at significant higher levels in TNM stage III tumours, positive correlated with node involvement [42]. In primary colorectal adenocarcinomas, elevated levels of expression for Bcl-xL and Bcl-w were reported to be associated with reduced expression of Bax [42]. Regarding the pro-apoptotic members of Bcl-2 family like Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk, increasing evidences suggest the involvement of Bak and Bax in the release of cytochrome *c*, based on phosphorylation of both Bak and Bax that facilitate their homo-oligomerization and subsequently the localization in mitochondria [29, 48]. Mutations in both

Bax and Bak genes confer cells the resistance to apoptosis [49, 50]. In colon cancer, Bax gene is frequently mutated in hereditary non-polyposis colorectal cancers [51] and microsatellite mutator phenotype [52, 53]. Decreased Bax expression was correlated with poor prognosis and progression towards metastasis [54].

One of the best known tumour suppressor proteins is **p53**, encoded by the tumour suppressor gene TP53 located on the short arm of chromosome 17 (17p13.1). The oncogenic property is due to a p53 mutations [55, 56], and half of all colorectal cancer cases show mutations in TP53 gene that were correlated with adenoma-to-carcinoma transitions and aggressive subsets of colorectal cancer [57, 58]. TP53 is a tumour suppressor gene in the mitochondrial apoptotic pathway, and one of the key regulators of cell-cycle control and apoptosis. Tumour cells presenting p53 mutations are defective in the induction of apoptosis. Its expression is down-regulated by survivin and Bcl-2 [59]. The molecular mechanisms that are employed by p53 to induce cell death in the context of suppressing cancer progression include the transcriptional regulation of pro-apoptotic PUMA expression, the generation of oxidative free radicals within mitochondrial components, the reduction of COX-2/PGE2 synthesis, and the induction of death receptor 5 [60, 61] (**Figure 1**).

Inhibitors of apoptosis (IAPs family) suppress apoptosis through inhibition of effector caspases [62]. The expression of inhibitor of apoptosis proteins (IAPs) is dysregulated in colorectal cancer. The anti-apoptotic regulators belonging to the IAP family, including XIAP, cIAP, and survivin, bind to caspase-3 and caspase-9 and thereby inhibit c aspase activity (**Figure 1**). Moreover, XIAP-associated factor 1 (XAF1) negatively regulates the anti-apoptotic function of XIAP. Molecules like c-FLIP, XIAP, cIAP2, and survivin have increased expression levels in colon cancer patients, and this has been correlated with disease progression and poor survival [47, 63, 64].

(b) Reduced caspase activity

During apoptosis process, the caspases implicated are either **initiator caspases** (e.g. caspase-2, caspase-8, caspase-9 and caspase-10) which are primarily responsible for the initiation of the apoptotic pathway, or **effector caspases** (caspase-3, caspase-6 and caspase-7), which are responsible in the actual cleavage of cellular components during apoptosis [65]. In the initiation and execution of apoptosis, caspases remain important players; therefore, low levels of caspases or impairment in caspase function may lead to a decrease in apoptosis and carcinogenesis [66, 67]. More than one caspase can be downregulated, contributing to colon cancer cell growth and development. Studies on differential expression by cDNA array showed a downregulation of both capase-8 and caspase-10, phenomenon that influences the pathogenesis of carcinomas [68].

(c) Impaired death receptor signalling

Several receptors and ligands that modulate the programmed cell death were described: TNF receptor superfamily, Fas/Fas-L, CD27, death receptors and ligands, receptors phosphatases. Signalling via death receptors could be impaired in human cancers via downregulation of

receptor surface expression as part of an adaptive stress response. Death receptors and their ligands are key players in the extrinsic pathway of apoptosis. The extrinsic signalling pathway leading to apoptosis involves transmembrane death receptors that are members of the tumour necrosis factor (TNF) receptor gene superfamily [69]. Several abnormalities in the death signalling pathways that can lead to evasion of the extrinsic pathway of apoptosis have been identified: the downregulation of receptor expression, the impairment of its function, as well as a reduced level in the death signals, all of which contribute to impaired signalling and a reduction of apoptosis. Reduced membrane expression of death receptors and abnormal expression of decoy receptors have also been reported to play a role in the evasion of the death signalling pathways in various cancers [12, 70] (**Figure 1**).

(d) Altered redox status in apoptosis induction

The oxidative stress process is characterized by an increased generation of reactive oxygen species (ROS) accompanied by a dysfunction of the antioxidant systems which exist in every cell, dependent on the metabolic state of the cell [71, 72]. The increased metabolic activity, mitochondrial dysfunction, peroxisome activity, oncogene activity, increased activity of oxidases, cyclooxygenases, lipoxigenases could be responsible for the generation and release of reactive oxygen species in tumour cells [73–75]. Low levels of ROS may influence processes like angiogenesis, cell proliferation and survival, while intermediate levels of ROS cause transient or permanent cell-cycle arrest and induce cell differentiation. When ROS production does not irreversibly alter cell viability, they can act as primary messengers, modulating several intracellular signalling cascades that lead to cancer progression [76]. High levels of ROS induce cell apoptosis or necrosis by causing an alteration of membrane permeability, a genetic instability, oxidative modifications that lead to less active enzymes or proteins more susceptible to proteolytic degradation [77]. Furthermore, ROS plays a crucial role in regulating expression of genes associated with cancer cell proliferation, angiogenesis, invasion and metastasis by activating transcription factors such as NF-κB, activator protein-1 (AP-1) and hypoxia inducible factor-1 (HIF-1α) [78].

Excessive production of ROS in tumour cells induces apoptosis or necrosis, and acts as an important inhibitor of cancer cell proliferation. Fas ligand mediates the induction of ROS, essential for the initiation of apoptotic signalling cascade and activation of the intrinsic apoptotic machinery by disruption of mitochondrial membrane integrity [79] (**Figure 1**). The transformed cells use ROS signals to drive proliferation to tumour progression. Tumour cells present an increased basal oxidative stress, making them vulnerable to chemotherapeutic agents that further augment ROS generation or weaken antioxidant defences of the cell [80]. Human colorectal tumours have increased levels of different markers of oxidative stress, such as ROS, nitric oxide (NO), lipid peroxides, glutathione peroxidase (GPx), catalase (CAT), and decreased cytosine DNA methylation [81–83].

ROS-sensitive signalling pathways are persistently elevated in many types of cancers, including colon cancer [84]. Reactive oxygen species can act as second messengers in cellular signalling. For example, hydrogen peroxide (H_2O_2) regulates protein activity through reversible oxidation of its targets, including protein tyrosine phosphatases, protein tyrosine kinases,

receptor tyrosine kinases and transcription factors [85, 86]. The mitogen-activated protein (MAP) kinase/Erk cascade, phosphoinositide-3-kinase (PI3K)/Akt-regulated signalling cascades, as well as the IκB kinase (IKK)/nuclear factor κ-B (NF-κB)-activating pathways are regulated by ROS. The extracellular signal-regulated kinase pathway (ERK) mediates signal transduction involved in cell proliferation, differentiation, and migration [87]. Activation of ERK in tumour cells by biocompounds (e.g. resveratrol, quercetin) results in anti-proliferative effects, such as apoptosis, senescence, or autophagy [88–91]. Then, ERK can activate apoptotic enzymes or phosphorylate transcription factors that regulate the expression of pro-apoptotic genes [92]. Cell death in tumour cells treated with resveratrol and quercetin was accompanied by increased ROS levels and p53 expression, decreased Bcl-2 expression, depolarization of the mitochondrial membrane, cleaved caspase-3, and DNA fragmentation [93]. Elevated levels of ROS triggered by treatment with biocompounds might inhibit dual-specificity phosphatases (DUSPs) that dephosphorylate and inactivate MAPKs, leading to ERK activation and promoting cancer cell death. Therefore, biocompounds might induce apoptosis in colon cancer cells via activation of the MEK/ERK pathway [94].

Mitochondrial release of H_2O_2 and NO upon apoptotic signals leads to the activation of **c**-Jun N-terminal kinases (JNKs). In response to ROS, JNKs catalyze the phosphorylation and downregulation of anti-apoptotic proteins such as Bcl-2 and Bcl-XL. JNK influences the composition of the Bax/Bcl-2 complex by increasing the expression of Bax, leading to the formation of Bax homodimers and dissipation of mitochondrial membrane integrity [95, 96]. In response to the increased generation of ROS, the MAPK family member p38MAP is also implicated in apoptotic signalling [97].

In addition, ROS play an important role in the regulation of IKK/NF-κB pathway. NF-κB is a redox-regulated sensor for oxidative stress that is activated by low doses of hydrogen peroxide. The activation of NF-κB is mediated through the NF-κB-inducing kinase (NIK) and IκB kinase (IKK) complexes. Degradation of IκB translocates NF-κB to the nucleus, where it acts as a transcription factor to induce the expression of anti-apoptotic and anti-inflammatory genes [98]. Peroxisome proliferator-activated receptor-gamma (PPARγ) has been shown to exert an inhibitory effect on cell growth in most cell types. The expression of PPARγ was significantly increased in tumour tissues from human colon cancer, and the occurrence of apoptosis induced by PPARγ ligands was sequentially accompanied by reduced levels of NF-κB and Bcl-2. PPARγ-Bcl-2 feedback loop might control the life–death continuum in colonic cells, while a deficiency in generation of PPARγ ligands could precede the development of human colon cancer [99].

4. Bioactive compounds and colon cancer

Recent studies focused on the discovery of new chemotherapeutic agents among natural products since many plants and their bioactive compounds displayed anti-carcinogenic and anti-proliferative effects towards colon cancer cells [13]. Positive correlations between antioxidant activities of plants and their anti-proliferative effects, suggesting the potential action of

antioxidants in inhibiting cancer cell growth, were also reported [13]. Among them, over 5000 flavonoids were found in vegetables and fruits, wines, seeds, nuts, grains and teas, herbs, and represent a class of plant secondary metabolites, known for their antioxidant properties [100]. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities [70]. The dietary compounds could interfere with specific stages of the carcinogenic process, inhibiting cell proliferation and inducing apoptosis in different types of cancer cells [101]. In addition, they might affect the expression of several detoxifying enzymes and their ability to modulate protein-signalling cascades [102].

4.1. Dietary sources and functional features

Since the 1950s, despite extensive clinical trials, mortality from colon cancer is a major public health problem in developed countries as a result of high consumption of animal fat or red meat and low intake of fibres or vegetables [103]. Protective factors include physical activity and increased intakes of dietary fibre, fish, nuts, dairy products, fruits and vegetables, while other factors, including weight and obesity, waist circumference, smoking, alcohol consumption, and red and processed meat intakes increase the risk of colorectal cancer [104, 105]. Using simple lifestyle modifications, changing the diet might substantially reduce the risk of colorectal cancer and could complement screening, so that CRC could be preventable in 90% of cases [106]. Over the last decade, different drugs and nutritional elements have been studied in preclinical as well as clinical trials and proved to have potential benefit in the field of CRC prevention [107]. Chemoprevention, the use of drugs or other agents to inhibit the development or progression of malignant changes in cells represents an alternative approach to reduce the mortality from colorectal cancer as well as other cancers [108].

Biocompounds	Source	Mechanisms of action	Refs.
Resveratrol	Grapes and red wine, mulberries, peanuts, seeds	Caspase activation NF-κB inhibition FasL induction Activation of MEK/ERK pathway Bcl-2 downregulation Increase of ROS and p53 levels	[92–94, 111, 137, 166, 167]
Genistein	Soybeans, fava beans, lupin, coffee	NF-κB inhibition Caspase activation Inhibition of PTK Inhibition of AKT pathway mdm2 downregulation	[113, 114]
Quercetin	Vegetables (capers, radish	Bcl-2, EGFR downregulation	[91–94,

Biocompounds	Source	Mechanisms of action	Refs.
	leaves, dill, cilantro, fennel, red onion, radicchio, kale), fruits (cranberry, black plums, blueberry, apples), seeds, nuts, tea, red wine	Cyclin D1, survivin inhibition Inhibition of Wnt/beta-catenin signalling pathway Increase of ROS and p53 levels Activation of MEK/ERK pathway	164]
Curcumin	Turmeric, curry, mustard	NF-κB inhibition ROS induction Modulation of MAPK pathway Downregulation of survivin and IGF-1 expression	[141–143, 146, 147]
Apigenin	Parsley, celery, dandelion, coffee, chamomile tea	Modulation of survival and death effectors (PI3K, AKT, ERK, STAT3, JNK, Mcl-1)	[119, 168]
Epigallocatechin gallate (EGCG)	Green tea, white tea, black tea	Modulation of ROS production NF-κB inhibition Inhibition of growth factor-dependent signalling (EGF, VEGF, IGF-I) Inhibition of MAPK and p21 pathways Downregulation of survivin	[148, 149, 151–155, 157, 176]
Silibinin	Milk thistle seeds	Bcl-2 downregulation Bax upregulation Decrease of cyclin D1 and c-myc expression Upregulation of death receptors DR4, DR5	[17, 127, 136, 138, 139]
Naringenin	Grapefruits, oranges and tomatoes (skin)	Losses in mitochondrial membrane potential Caspase-3 activation Intracellular ROS production Sustained ERK activation	[129, 163]
Pomegranate juice	Pomegranate	Downregulation of Bcl2-XL Caspase-3 and caspase-9 activation NF-κB inhibition Suppression of AKT pathway	[131, 132, 158, 160]

Biocompounds	Source	Mechanisms of action	Refs.
Sulforaphane	Broccoli, Brussels sprouts, cabbage, cauliflower, kale, collards, kohlrabi, mustard, turnip, radish, arugula, watercress	Upregulation of Bax, p21 G_2/M cell-cycle arrest	[121, 122, 177, 178]
Ellagic acid	Strawberries, walnuts, pecans	Disruption in mitochondrial membrane potential Activation of caspase-3, caspase-8 and caspase-9 Inactivation of PI3K/Akt pathway Bax upregulation; Bcl-2 downregulation Increase of ROS production	[169, 170]
Lycopene	Tomatoes, red carrots, watermelons, papayas	Bax and FasL upregulation; Bcl-2 and Bcl-XL downregulation Downregulation of Akt, NF-κB	[171, 172]

Table 1. Biocompounds—dietary sources and mechanisms of action involved in modulation of apoptosis.

Different natural compounds display a wide range of biological activities, including anti-inflammatory and cytoprotective activities, and several are known to act as anti-cancer reagents. Curcumin from turmeric, genistein from soybean, tea polyphenols like epigallocatechin gallate from green tea, resveratrol from grapes, sulforaphane from broccoli, isothiocyanates from cruciferous vegetables, silymarin from milk thistle, diallyl sulphide from garlic, lycopene from tomato, rosmarinic acid from rosemary, apigenin from parsley, gingerol from ginger and quercetin (**Table 1**) have high antioxidant activities, and demonstrated anti-proliferative effects against various cancer cell lines [13].

Resveratrol (RSV, trans-3,4′,5-trihydroxystilbene), a naturally occurring polyphenol phytoalexin, is abundant in a wide variety of plants and their products, including grapes and red wine, mulberries, peanuts, seeds, and has anti-inflammatory, antioxidant, anti-neoplastic, anti-carcinogenic, anti-tumorigenic, cardioprotective, neuroprotective, anti-aging and antiviral effects [102, 109]. Resveratrol exhibited anti-colon cancer properties by inhibiting cell proliferation, inducing apoptosis, decreasing angiogenesis, and causing cell-cycle arrest [110, 111].

Genistein (GST, 4′,5,7-trihydroxyisoflavone) is a natural compound found in lupin, fava beans, soybeans, coffee and occurs in Asian diet, rich in soy products [112]. It is a strong topoisomerase inhibitor, similarly to etoposide and doxorubicin. It has a wide spectrum of activity, expressed in protecting cells from malignant transformation, reducing proliferation of tumour cells and stimulating apoptosis [113, 114].

Quercetin (QCT, 3,3′,4′,5,7-pentahydroxyflavone) is an important dietary flavonoid, which presents in different vegetables (e.g. capers, radish leaves, dill, cilantro, fennel, red onion, radicchio, kale), fruits (cranberry, black plums, blueberry, apples), seeds, nuts, tea and red wine. It is involved in suppression of tumour-related processes, including oxidative stress, proliferation and metastasis. QCT has also received greater attention as pro-apoptotic flavonoid with a specific and almost exclusive activity on tumour cell lines rather than normal, non-transformed cells [115]. The anti-tumour effect found in SW480 colon cancer cell line was related to the inhibition of Cyclin D (1) and survivin expression, as well as Wnt/beta-catenin signalling pathway [116].

Curcumin (CRM, 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a diarylheptanoid and the principal curcuminoid of turmeric, extracted from *Curcuma longa*; it possesses anti-inflammatory and antioxidant properties, and has a strong inhibitory effect on cell proliferation in the HT-29 and HCT-15 human colon cancer cell lines [117, 118].

Apigenin (APG, 4′,5,7-trihydroxyflavone) is one of the most common flavonoids widely distributed in fruits and vegetables, such as parsley, celery, dandelion coffee and chamomile tea. However, apigenin only showed a modest anti-tumour activity towards cancer cells. New strategies are needed to enhance apigenin's anti-tumour efficacy [119].

Epigallocatechin 3-O-gallate (EGCG) [(2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate] is the most abundant catechin in tea, a polyphenol found in high quantities in the dried leaves of white tea, green tea and, in smaller content in black tea [120]. EGCG was found to exert profound anti-inflammatory, antioxidant, anti-infective, anti-cancer, anti-angiogenic, and chemopreventive effects [120].

Sulforaphane (1-Isothiocyanato-4-methylsulfinylbutane) is found in cruciferous vegetables such as broccoli, Brussels sprouts, cabbage, cauliflower, bok choy, kale, collards, Chinese broccoli, broccoli raab, kohlrabi, mustard, turnip, radish, arugula and watercress. Young sprouts of broccoli and cauliflower are particularly rich in glucoraphanin. It is produced when the enzyme myrosinase transforms glucoraphanin, a glucosinolate, into sulforaphane upon damage to the plant which allows the two compounds to mix and react [121]. Sulforaphane (SFN) is a naturally occurring chemopreventive agent, inducing the cell-cycle arrest and apoptosis in colon cancer. However, little is known about the differential effects of SFN on colon cancer and normal cells [122].

Lycopene is a bright carotenoid pigment and phytochemical found in tomatoes, red carrots, watermelons and papayas. Although lycopene is chemically a carotene, it has no vitamin A activity. Also foods that are not red may contain lycopene, such as asparagus or parsley. Lycopene exhibited potential anti-carcinogenic activity, and the consumption of tomatoes was associated with reduced risk of several types of human cancer, including colon cancer [123, 124].

Glucobrassicin is a type of glucosinolate found in cabbages, broccoli, mustards, horseradish and woad. The main hydrolysis product after glucobrassicin is degraded by myrosinase is indole-3-carbinol, which was found to have apoptosis-inducing effects in a concentration- and time-dependent manner in human colon cancer cells [125, 126].

Silibinin is the major active constituent of **silymarin**, a standardized extract of the milk thistle seeds, that contains a mixture of flavonolignans and was shown to induce apoptosis in colon cancer cells [127, 128].

Naringenin (5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one) is a flavanone, considered to have a bioactive effect on human health as antioxidant, free radical scavenger, anti-inflammatory, carbohydrate metabolism promoter and immune system modulator. It can be found in grapefruits, oranges and tomatoes (skin) [129].

Pomegranate juice obtained from Punica granatum is rich in polyphenol compounds such as gallo, ellagitannin and flavonoid classes. It possesses therapeutic activity such as anti-atherogenic, anti-parasitic, anti-microbial, antioxidant, anti-carcinogenic and anti-inflammatory effects [130]; in preclinical animal studies, oral consumption of pomegranate extract inhibited the growth of lung, skin, colon and prostate tumours [131]. It was shown that pomegranate juice derivatives promote apoptosis of colon cancer cells by inducing the intrinsic pathway, but no effect was shown on the extrinsic pathway [132].

5. Bioactive compounds and their role in modulation of apoptosis in colon cancer

Results of clinical trials revealed that colon cancer can be successfully treated by chemotherapy, if the tumour selective detection can be substantial increased. In this regard, there is an increasing demand for biomarkers for risk assessment, early detection, prognosis and surrogate end points. This will be possible by the introduction of new drugs with more precise mechanisms of action, such as those acting specifically upon well-known aberrant pathways (e.g. apoptosis, cell signalling) [133]. New drugs can initiate or modulate the apoptosis cascade acting on caspases, Fas, Bax, Bid, APC or molecules which promote colon cancer cell survival (p53 mutants, Bcl-2 or COX-2) [134].

The implementation of new treatment options (and the management of metastatic colon cancer) must take into account the role of apoptosis in colon tumorigenesis with a highlight on the mechanisms leading to chemotherapeutic resistance as well as immune system evasion [69]. From this point of view, apoptosis can be considered as a potential target for cancer treatment at various stages of tumour progression, while chemoprevention as well as the apoptotic mechanisms could be utilized in the prevention and management of tumorigenesis [35, 135].

5.1. Mechanisms and targets involved

Grape seed extract that consists in a mixture of polyphenols was able to decrease cyclin D1, and c-myc expression, preventing cycle cell disruption, reduce expression of iNOS and COX-2 decreasing oxidative cellular stress [136]. On the other hand, *in vitro* studies performed on CRC cell lines showed that grape seed extract induces apoptosis via activation of caspase-3,

http://dx.doi.org/10.5772/63382

caspase-8, and caspase-9, and also generation of ROS. It is worth mentioning that proapoptotic activity of grape seed extract has no effect in normal colonocytes [107].

Resveratrol has anti-CRC effects by inhibiting tumour initiation and progression by affecting caspase activation, NF-κB inhibition and FasL induction. Resveratrol could suppress inflammatory responses through decreasing nitric oxide levels and inhibiting the phosphorylation of the IκB enzyme complex, thus suppressing the activation of NF-κB dependent mechanisms [111, 137]. It was also described to interfere with mitochondrial functions by inhibiting mitochondrial ATP synthesis through its binding to F_1-ATPase. In addition, resveratrol can antagonize anti-apoptotic proteins that prevent the induction of apoptosis in cancer cells. Resveratrol induces p53-independent upregulation of p21, p21-triggered cell-cycle arrest and subsequently cell-cycle-dependent depletion of the anti-apoptotic protein survivin, thereby sensitizing cancer cells to TRAIL-induced apoptosis. Moreover, it suppresses expression levels of additional anti-apoptotic proteins (e.g. Bcl-x_L and MCL-1). The anti-tumour activities of resveratrol are also due to its ability to interfere with the phosphatidylinositol-3 kinase (PI-3K)/AKT and the MAPK pathways [13] (**Table 1**).

Silibinin: Studies *in vitro* and *in vivo* have shown a chemopreventive role in CRC by interfering in proliferation, signalling pathway and inflammation processes [17]. It was also found to cause decrease of cyclin D1 and c-myc expression [127]. Moreover, silibinin modulates the expression of anti-apoptotic proteins (Bcl-2, Mcl-1, X-linked inhibitor of apoptosis protein, and survivin) [136]. Silibinin can induce apoptosis by downregulation of the anti-apoptotic protein Bcl-2 and upregulation of the pro-apoptotic protein Bax, inverting the Bcl-2/Bax ratio. Sibilin also promotes apoptosis by upregulating transcription of the death receptors DR4 and DR5 [138], inhibits TNF-α activation of NF-κB, decreases expression of COX-2 and iNOS [139]. Silibinin decreased the expression of IL-1, TNF-alpha and their downstream target MMP7, all of them being upregulated during colon carcinogenesis [127, 138]. In a study evaluating silibinin pharmacodynamics, Hoh et al. [140] showed that silibinin is not toxic to normal colonic epithelium (**Table 1**).

Curcumin is effective in apoptosis triggering, inhibiting DNA mutations, cancer cell proliferation, metastasis and inflammation. Curcumin induces the production of ROS in concentration that leads to p21 protein upregulation, and consequently inhibiting cancer cell growth [141]. Moreover, curcumin interferes with the protein kinase (MAPK) pathway, which in turn decreases production of TNF-α and COX-2 as well as downregulates the expression of NF-κB and IL-6 [142, 143]. The downregulation of NF-κB levels has also effect on expression level of c-myc, cyclin D1 and Bcl-2 genes, and finally modulates the cell cycle [144]. It promotes cancer cell apoptosis by inducing expression of proapoptotic proteins (Bax, Bim, Bak, Noxa) and inhibiting expression of anti-apoptotic proteins (Bcl-2, Bcl-xl) [143]. Curcumin prevents the formation of metastases by decreasing vascular endothelial growth factor (VEGF) and matrix metalloproteinase 9 expression [144]. In recent in vitro study in CRC cells, Patel et al. [145] have shown that curcumin inhibits the receptor expression of HER2 and insulin-like growth factor 1 (IGF-1) which is well known to create resistance to 5-fluorouracil and oxaliplatin. Curcumin downregulates the expression of survivin and IGF-1 by activating the expression of p53 and reducing TNF-α levels, leading to activation of apoptotic signal [146, 147] (**Table 1**).

Epigallocatechin 3-Gallate Epigallocatechin 3-gallate (EGCG) has a strong antioxidant activity preventing ROS formation, blocking cancer cell proliferation and metastasis formation by down-regulating the expression of growth factors (epidermal growth factor, IGF-1, VEGF) [148]. EGCG blocks the cell cycle through the modulation of both MAPK and p21 pathways [149]. Furthermore, by upregulation of p53, EGCG induces apoptosis in CRC cells [150]. EGCG promotes cell growth arrest and induces apoptosis by affecting regulatory proteins of the cell cycle and inhibition of NFκB [151–153]. Some reports point out that the ROS-related effects may contribute to the anti-proliferative and pro-apoptotic activity of EGCG [154]. The effects are associated with modulation of reactive oxygen species (ROS) production. Although EGCG has a dual function of antioxidant and pro-oxidant, EGCG-mediated modulation of ROS production is reported to be responsible for its anti-cancer effects. The EGCG-mediated inhibition of NF-κB signalling is also associated with inhibition of migration, angiogenesis and cell viability [155]. Furthermore, it inhibits growth factor-dependent signalling (e.g. EGF, VEGF and IGF-I), the MAPK pathway, proteasome-dependent degradation and expression of COX-2. EGCG seems to directly interact with and modulate the character of membrane lipid rafts, which explains the ability to alter signalling processes of growth factor receptors. Furthermore, EGCG inhibits telomerase, topoisomerase II and DNA methyltransferase 1, thereby affecting the functions of chromatin [153, 156]. EGCG has a protective effect against NO-induced apoptosis in HDPC by scavenging ROS and modulating the Bcl-2 family [157] (**Table 1**).

Pomegranate may inhibit cancer cell proliferation and apoptosis through the modulation of cellular transcription factors and signalling proteins. In previous studies, pomegranate juice and its derivate inhibited proliferation and induced apoptosis in colon cancer cells, significantly suppressed TNF-α-induced COX-2 protein expression. It also reduced phosphorylation of the p65 subunit and binding to NF-κB, and abolished TNF-α induced AKT activation, playing an important role in the modulation of cell signalling in colon cancer cells [158]. The pomegranate juice exhibited a dose- and time-dependent decrease in cell proliferation, inducing cell-cycle arrest in the G_0/G_1 and G_2/M stages of the cell cycle, followed by apoptosis [159]. In the same regard, Larrosa et al. [160] have shown that induction of apoptosis was due to the downregulation of Bcl2-XL protein as well as activation of caspase-3 and caspase-9, but not caspase-8 (**Table 1**).

Citrus flavonoids: Volatile oil of *Citrus aurantifolia*, showed 78% growth inhibition of human colon cancer cells, induced the characteristic pattern of DNA fragmentation, via caspase-3 dependent pathway along with modulation of apoptosis-related protein expression [161].

Orange (*Satsuma mandarin*) juice contains a lot of flavonoids, which are potential chemoprotective compounds, with the capacity to suppress the expression of several cytokines (TNF-α, IL-1β, IL-6) as well as inflammatory enzymes (COX-2 and iNOS). In human colon cancer cell lines, the mechanism that induced the inhibition of their growth acted by blocking the cell cycle in G_0/G_1 phase and reducing levels of cyclins (A, D1 and E) [162].

Naringenin: Treatment with naringenin derivates resulted in significant apoptosis-inducing effects concomitant with losses in mitochondrial membrane potential, caspase activation, intracellular ROS production and sustained ERK activation [129]. In human colon adenocar-

cinomas, it induced activation of p38/MAPK, leading to the pro-apoptotic caspase-3 activation and poly (ADP-ribose) polymerase cleavage [163] (**Table 1**).

Quercetin plays a role in inhibiting tumorigenesis in colon cells through antioxidant, anti-inflammatory, anti-proliferative and pro-apoptotic mechanisms. Quercetin downregulated Bcl-2 through the inhibition of NF-κB and inhibited phosphorylation of EGFR suppressing downstream signalling in colon carcinoma cells. It augments TRAIL-induced apoptotic death and inhibits cyclin D1, survivin expression and Wnt/beta-catenin signalling pathway [91, 164] (**Table 1**).

Apigenin: Studies have shown that apigenin induces cell-cycle arrest and causes apoptosis in different cancer cells including colon cancer through modulation of various survival and death effectors, such as PI3K, AKT, ERK, STAT3, JNK and Mcl-1 [119] (**Table 1**).

Genistein inhibits the activation of NF-κB and Akt signalling pathways, both of which are known to maintain a homeostatic balance between cell survival and apoptosis; antagonizes estrogen- and androgen-mediated signalling pathways in the processes of carcinogenesis [114]. It has antioxidant properties, being a potent inhibitor of angiogenesis and metastasis. Genistein is a known inhibitor of protein-tyrosine kinase (PTK), which may attenuate the growth of cancer cells by inhibiting PTK-mediated signalling mechanisms. Genistein also inhibits topoisomerase I and II, 5α-reductase and protein histidine kinase, all of which may contribute to the anti-proliferative or pro-apoptotic effects [113]. It also down-regulates mdm2 at both transcriptional and post-translational levels [165] (**Table 1**).

5.2. Epigenetic mechanisms related to apoptosis, influenced by natural compounds

Several papers pointed the role of various natural compounds that target the **epigenetic mechanisms** in order to modulate the biologic activities, including apoptosis [173]. Epigenetic control mechanisms are reversible and natural compounds that target them may contribute to the development of new and attractive therapeutic strategies. A review of some natural compounds that target apoptosis through epigenetic mechanisms is presented below.

EGCG has a role in inhibiting histone deacetylases (HDACs) [174]. Site-specific acetylation of histones is essential to switch between permissive and repressive chromatin structures. Chromatin remodelling allows the regulation of gene expression, and it takes place under the action of enzymes responsible for acetylation (histone acetyl transferase, HAT) and deacetylation (histone deacetyl transferase, HDAC) [175]. An increased HDAC activity is characteristic to many cancers and is associated with alterations of several cellular mechanisms, including apoptosis. Therefore, by targeting HDACs, it is possible to modulate several cell processes like cell cycle, cell differentiation and apoptosis. Combined treatments of EGCG and sodium butyrate (NaB), in physiologically achievable concentrations, were shown to promote apoptosis and induce cell-cycle arrest in RKO, HCT-116 and HT-29 colon cell lines [176]. Both EGCG and NaB are epigenetic regulators, and treatment with these two compounds reduced both the expression of survivin (which has anti-apoptotic activity), as well as the expression of enzymes involved in epigenetic regulation (HDAC and DNA methyltransferase- DNMT) [176].

HDAC activity is also inhibited by **sulforaphane** (SFN) and induces an increased histone acetylation, mainly at p21 and Bax 2 promoters, events that lead to G_2/M cell-cycle arrest and apoptosis [177]. Oral administration of SFN in mice increased p21 expression via HDAC inhibition, while in APC-knock-out mice the same treatment leaded to specific increase of acetylation in H3 and H4 with decrease of intestinal polips [178]. Moreover, SFN was found to inhibit DNMTs (enzymes responsible for DNA methylation) in CaCo-2 colon cancer cell line [179]. Another epigenetic mechanism used by SFN to exert its activity is through miRNAs: in NCM460 and NCM356 normal colon epithelial cells SFN upregulated 15 miRNAs, among which several were involved in apoptosis (miR-9, miR-135b) [180].

Resveratrol activates type III HDAC inhibitors, sirtuin 1 (SIRT1) and p300 [181] which in turn negatively modulates the expression of survivin. On the other hand, in colon cancer cells, resveratrol inhibits the cell growth and induces apoptosis through miRNAs [182, 183]. Combined treatment of quercetin and resveratrol induced apoptosis in colorectal cancer cells through downregulation of miR-27a [184].

6. Combined therapy of colon cancer: current strategies and future directions

Conventional therapeutic approaches, including chemotherapy, radiotherapy and surgery, are limited for the treatment of advanced colon cancer, in prevention of the disease recurrence, and are associated with a high risk of complications, highlighting the need to develop new therapeutic strategies. The majority of CRC patients receive chemotherapy using multiple agents that are currently approved for the treatment in the appropriate setting, but many patients have tumours intrinsically resistant to them. However, it is a complex process to select the optimal chemotherapy for each patient, and the difference between theory and practice is still a problem. That's why new concepts and modern technologies are promoted by precision medicine in order to achieve a personalized treatment for cancer patients.

6.1. RTCA: useful tool for screening biocompounds and drugs

Contrast data are available on the anti-cancer effects of biocompounds in colon cancer, whether they could influence the effects of oncolytic drugs against the cell growth and apoptosis of human colon cancer cells, or which might be the proper concentrations of the compounds with cytotoxic or cytostatic potential. Real-time impedance data obtained by the xCELLigence System (ACEA Biosciences) might be used to generate compound-specific profiles which are dependent on the biological mechanisms of action of each compound used. The actual kinetic response of the cells within an assay prior or subsequent to certain manipulations provides important information regarding the biological status of the cell, such as cell growth, cell arrest, morphological changes and apoptosis [185]. Changes in a cell status, such as cell morphology, cell adhesion or cell viability lead to a change in cell index (CI), which is a quantitative measure of cell number present in a well. For example, the cytotoxicity versus proliferative capacity of genistein, resveratrol or 5-fluorouracil was assessed in LoVo colon cancer cell line in order to

http://dx.doi.org/10.5772/63382

modulate the chemosensitivity of colon cancer cells to drug treatment, and overcome the chemo-resistance. The entire length of the assay was presented, allowing informed decisions regarding the timing of certain manipulations or treatments, choice of the proper concentrations for further end-point assays, such as flow-cytometry techniques or molecular biology approaches [186, 187].

6.2. Modulation of apoptosis by combined therapy

Many anti-cancer drugs act during physiological pathways of apoptosis, leading to tumour cell destruction. By combining natural compounds with anti-cancer drugs, an increase of the effects might be obtained, specifically in highly invasive cancer cells, while in non-tumoral cells the natural compounds could reduce the cytotoxic side effects [115] (**Figure 2**).

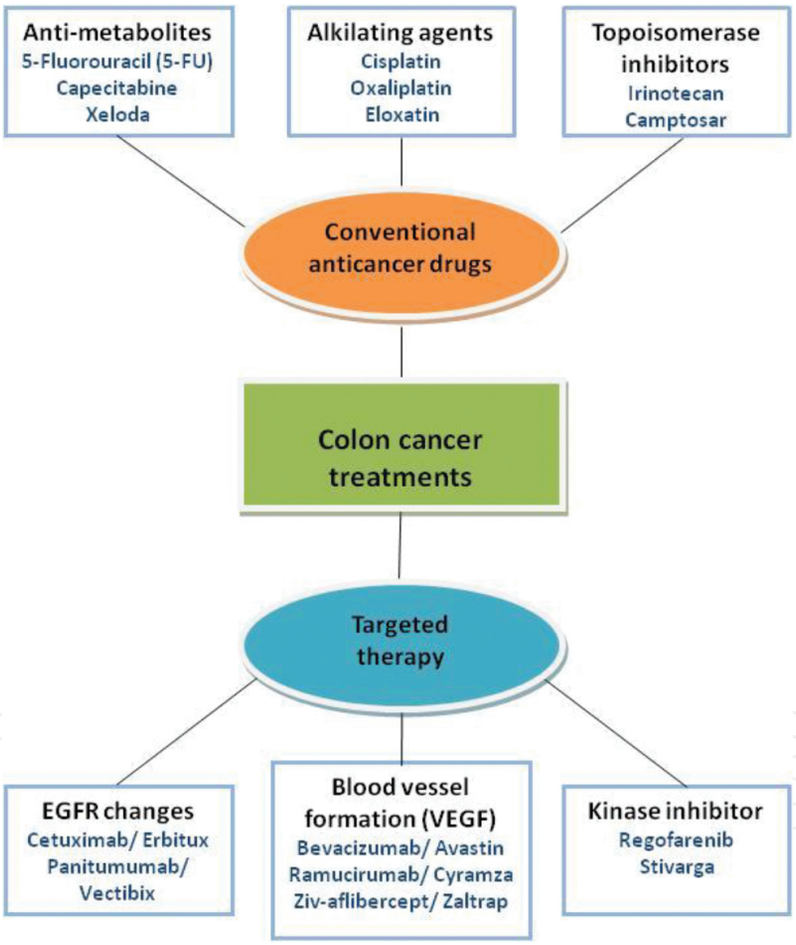

Figure 2. Available colon cancer therapeutic agents with disparate mechanisms of action.

A wide variety of currently available cancer therapeutical agents (**Figure 2**), with disparate mechanisms of actions, lead to the same mode of cell death [188]:

a. 5-Fluorouracil (5-FU) that blocks the thymidylate synthase (TS), which is essential for DNA synthesis;

b. Capecitabine that blocks thymidylate synthase (orally administered prodrug converted to 5-FU)

c. Oxaliplatin that inhibits DNA replication and transcription by forming inter- and intra-strand DNA adducts/cross-links;

d. Irinotecan that inhibits topoisomerase I, an enzyme that facilitates the uncoiling and recoiling of DNA during replication;

e. Bevacizumab, a monoclonal antibody, which binds to vascular endothelial growth factor (VEGF) ligand;

f. Cetuximab, a monoclonal antibody to epidermal growth factor receptor (EGFR) (chimeric), that blocks the ligand-binding site;

g. Panitumumab, a monoclonal antibody to EGFR (fully humanized), that blocks the ligand-binding site [188].

Several anti-cancer drugs act during physiological pathways of apoptosis, leading to tumour cell destruction [23, 189]. The pattern and extent of the cell damage induced by chemotherapeutics, like fluoropyrimidines, in human cancer cells have been suggested to depend also on the pathways downstream from drug-target interactions that once triggered will initiate programmed cell death (apoptosis) [190, 191]. 5-Fluorouracil (5-FU) is one of the widely used chemotherapeutic drugs targeting various cancers, but its chemoresistance remains as a major obstacle in clinical settings. Several groups reported the induction of apoptosis by 5-fluorouracil (5-FU) in HT29 [192] or LoVo human colon cancer cell lines [186, 187]. The long exposure of colon cells to 5-FU treatments influences both pro- and anti-apoptotic molecules like P53 and Bax, or Bcl-2 and Bcl-XL [193]. Several studies showed that 5-FU inhibits DNA proliferation in colon cancer cells by inhibiting the enzyme thymidylate synthase, leading to apoptosis, a mechanism of active cell death characterized by rapid loss of plasma membrane integrity, DNA fragmentation and altered expression of numerous genes [46, 52, 104] (**Figure 2**).

The biocompounds extracted from botanicals may be used as chemopreventive and therapeutic agents for various human cancers, inclusive colon cancer [94]. The active biocompounds might induce cancer-selective cell death by increasing production of reactive oxygen species. The cancer cells have increased levels of ROS accompanied by a highly active antioxidant defense system; therefore, the tumour cells are unable to recover from additional oxidative stress and die. It is accepted that mitochondria-derived ROS play a critical role in their pro-death and chemopreventive responses. The natural biocompounds inhibit mitochondrial electron transport chains causing ROS production, thus triggering apoptotic cell death [80]. By combining flavonoids with anti-cancer drugs, it might be obtained an increase of the desired effects, specifically in highly invasive cancer cells, while in non-tumour cells the cytotoxic side effects could be reduced [186]. In vitro, studies showed that LoVo colon cancer cells were markedly sensitized to apoptosis by both 5-FU and genistein compared to the 5-FU treatment alone. When time of incubation was increased, treatments with GST and/or 5-FU had much stronger effects on the induction of apoptosis in LoVo cells, evaluated by using annexin-V/FITC and PI double staining, followed by flow-cytometry analysis [186]. Similar studies

demonstrated the additive effect of GST to anti-cancer drug treatment, and in reversing the multi-drug resistance [13, 14].

Experimental assays showed that resveratrol (RSV) induced higher levels of early and late apoptosis compared to untreated or 5-FU-treated LoVo cells. When treatments were prolonged to 72 h, stronger effects were observed both for RSV alone and combined treatments with 5-FU [187]. Flow-cytometry analyses showed that treatments with 25 μM 5-FU or 50 μM RSV slightly increased the expression of the pro-apoptotic molecules p53 and Bax. The combined treatments of 50 μM RSV and 25 μM 5-FU induced a higher increase of p53 expression compared to the non-treated cells. Also the increase of Bax expression was much higher for the combined treatments compared to non-treated cells or treated cells with 5-FU alone. Both RSV and 5-FU treatments seemed to decrease Bcl-2 expression, but the effect was stronger for the combined treatments. Combined treatments induced a higher increase of pro-apoptotic antigen expression, both for P53 and Bax, compared to 5-FU treatment [187].

Therefore, addition of flavonoids and other natural compounds might be an alternative approach in order to obtain the same or a stronger anti-tumour response, enhance the chemosensitivity of tumours to anti-cancer drugs, or diminish the undesirable side effects by using lower concentrations [194, 195].

7. Conclusions

A big challenge for a successful treatment of colon cancer is the lack of eradication of the entire tumour cell population and the consequent development of chemoresistance. Since many anti-cancer drugs act during physiological pathways of apoptosis, leading to tumour cell destruction, elucidation of the mechanisms that govern the programmed cell death process seems to be of great importance for carcinogenesis, tumour evasion, and to have practical implications for anti-cancer therapy. Many therapeutic drugs used in cancer treatment proved to induce resistance in cancer cell killing, and their number are rapidly increasing, possibly through the modulation of survival cell components such as proliferative or anti-apoptotic proteins. Contrast data are available on the anti-cancer effects of natural compounds in colon cancer, whether they could influence the effects of oncolytic drugs against the growth and apoptosis of human colon cancer cells, or which might be the proper concentrations of compounds with cytotoxic or cytostatic potential. From a large number of natural compounds investigated, several have been shown to be promising, based on their anti-cancer effects related to apoptosis. A newly arising field involves therapeutic approaches in cancer in order to induce tumour cells to undergo apoptosis and break the cancer cell resistance to apoptosis commands. Therefore, manipulation of the mechanisms of programmed cell death process could be of outstanding importance for malignant transformation, and alternative approaches might be used to obtain a stronger anti-tumour response, and/or diminish the undesirable side effects by using lower concentrations of anti-cancer drugs. Thus, new concepts and modern technologies are promoted by precision medicine in order to achieve a personalized treatment for cancer patients.

Author details

Marinela Bostan[1], Mirela Mihaila[1], Camelia Hotnog[1], Coralia Bleotu[2], Gabriela Anton[3], Viviana Roman[1] and Lorelei Irina Brasoveanu[1*]

*Address all correspondence to: luli_brasoveanu@yahoo.com

1 Center of Immunology Dept., "Stefan S. Nicolau" Institute of Virology, Bucharest, Romania

2 Cellular and Molecular Pathology Dept., "Stefan S. Nicolau" Institute of Virology, Bucharest, Romania

3 Molecular Virology Dept., "Stefan S. Nicolau" Institute of Virology, Bucharest, Romania

References

[1] Jemal, A., R. Siegel, E. Ward, Y. Hao, J. Xu, T. Murray (2008) Cancer statistics. *CA Cancer J Clin*. 58:71–96.

[2] Weber, G.F. Molecular mechanisms of cancer. Dordrecht, The Netherlands: Springer; 2007. 645 p. (p. 39, 45–54, 93–99).

[3] Desoize, B., J. Jardillier (2000) Multicellular resistance: a paradigm for clinical resistance? *Crit Rev Oncol Hematol*. 36(2–3):193–207.

[4] Gottesman, M.M. (2002) Mechanisms of cancer drug resistance. *Annu Rev Med*. 53:615–627.

[5] Zimmermann, K.C., C. Bonzon, D.R. Green (2001) The machinery of programmed cell death. *Pharmacol Ther*. 92:57–70.

[6] Townson, J.L., G.N. Naumov, A.F. Chambers (2003) The role of apoptosis in tumor progression and metastasis. *Curr Mol Med.* 3:631–42.

[7] Miura, K., W. Fujibuchi, K. Ishida, T. Naitoh, H. Ogawa, T. Ando, N. Yazaki, K. Watanabe, S. Haneda, C. Shibata, I. Sasaki (2011) Inhibitor of apoptosis protein family as diagnostic markers and therapeutic targets of colorectal cancer. *Surg Today.* 41:175–182.

[8] Meadows, G.G. (2012) Diet, nutrients, phytochemicals, and cancer metastasis suppressor genes. *Cancer Metastasis Rev*. 31(3–4):441–454.

[9] Mattern, J. (2003) Drug resistance in cancer: a multifactorial problem. *Anticancer Res.* 23(2C):1769–1772.

[10] De Angelis, P.M., D.H. Svendsrud, K.L. Kravik, T. Stokke (2006) Cellular response to 5-fluorouracil (5-FU) in 5-FU-resistant colon cancer cell lines during treatment and recovery. *Mol Cancer.* 5:20–45.

[11] Gerhauser, C. (2013) Cancer chemoprevention and nutri-epigenetics: state of the art and future challenges. *Top Curr Chem.* 329:73–132.

[12] Fulda, S. (2010) Evasion of apoptosis as a cellular stress response in cancer. *Int J Cell Biol.* 2010; 2010:370835–370841.

[13] Fulda, S. (2010) Modulation of apoptosis by natural products for cancer therapy. *Planta Med*. 76:1075–1079.

[14] Shu, L., K.L. Cheung, T.O. Khor, C. Chen, A.N. Kong (2010) Phytochemicals: cancer chemoprevention and suppression of tumor onset and metastasis. *Cancer Metastasis Rev*. 29:483–502.

[15] Thornthwaite, J.T., H.R. Shah, P. Shah, W.C. Peeples, H. Respess (2013) The formulation for cancer prevention & therapy. *Adv Biol Chem.* 3:356–387.

[16] Russo, M., C. Spagnuolo, I. Tedesco, G.L. Russo (2010) Phytochemicals in cancer prevention and therapy: truth or dare? *Toxins.* 2:517–551.

[17] Rajamanickam, S., R. Agarwal (2008) Natural products and colon cancer: current status and future prospects. *Drug Dev Res.* 69(7):460–471.

[18] Fox, J.T., S. Sakamuru, R. Huang, N. Teneva, S.O. Simmons, M. Xia, R.R. Tice, C.P. Austin, K. Myung (2012) High-throughput genotoxicity assay identifies antioxidants as inducers of DNA damage response and cell death. *Proc Natl Acad Sci. USA* 109(14): 5423–5428.

[19] Watson, A.J.M. (2004) Apoptosis and colorectal cancer. *Gut*. 53:1701–1709.

[20] Pritchard, C.C., W.M. Grady (2011) Colorectal cancer molecular biology moves into clinical practice. *Gut.* 60(1):116–29.

[21] Tejpar, S., M. Bertagnolli, F. Bosman, H.J. Lenz, L. Garraway, F. Waldman, R. Warren, A. Bild, D. Collins-Brennan, H. Hahn, D.P. Harkin, R. Kennedy, M. Ilyas, H. Morreau, V. Proutski, C. Swanton, I. Tomlinson, M. Delorenzi, R. Fiocca, E. Van Cutsem, A. Roth (2010) Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. *Oncologist.* 15(4):390–404.

[22] Samowitz, W.S. (2008) Genetic and epigenetic changes in colon cancer. *Exp Mol Pathol.* 85(1):64–67.

[23] Kim, R., K. Tanabe, Y. Uchida, M. Emi, H. Inoue, T. Toge (2002) Current status of the molecular mechanisms of anticancer drug-induced apoptosis. The contribution of molecular-level analysis to cancer chemotherapy. *Cancer Chemother Pharmacol*. 50:343–352.

[24] Kaufmann, S.H., D.L. Vaux (2003) Alterations in the apoptotic machinery and their potential role in anticancer drug resistance. *Oncogene*. 22(47):7414–7430.

[25] Negrini, S., V.G. Gorgoulis, T.D. Halazonetis (2010) Genomic instability an evolving hallmark of cancer. *Nat Rev Mol Cell Biol.* 11(3):220–228.

[26] Su, Z., Z. Yang, Y. Xu, Y. Chen, X. Yu (2015) Apoptosis, autophagy, necroptosis, and cancer metastasis. *Mol Cancer.* 14:48.

[27] Elmore, S. (2007) Apoptosis: a review of programmed cell death. *Toxicol Pathol.* 35(4): 495–516.

[28] Taylor, R.C., S.P. Cullen, S.J. Martin (2008) Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol.* 9(3):231–241.

[29] Hassan, M., O. Feyen, E. Grinstein (2009) Fas-induced apoptosis of renal cell carcinoma is mediated by apoptosis signal-regulating kinase 1 via mitochondrial damage-dependent caspase-8 activation. *Cell Oncol*. 31(6):437–456.

[30] Mendelsohn J., P.M. Howley, M.A. Israel, J.W. Gray, C.B. Thompson, editors. The Molecular Basis of Cancer. 4th ed. Philadelphia (PA): Elsevier; 2015. 863 p.

[31] Yang, S.Y., K.M. Sales, B. Fuller, A.M. Seifalian, M.C. Winslet (2009) Apoptosis and colorectal cancer: implications for therapy. *Trends Mol Med.* 15:225–233.

[32] Giansanti, V., A. Torriglia, A.I. Scovassi (2011) Conversation between apoptosis and autophagy: is it your turn or mine? *Apoptosis.* 16(4):321–333.

[33] Danial, N.N., S.J. Korsmeyer (2004) Cell death: critical control points. *Cell.* 116:205–219.

[34] Locksley, R.M., N. Killeen, M.J. Lenardo (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell.* 104(4):487–501.

[35] Ashkenazi, A., V.M. Dixit (1998) Death receptors: signaling and modulation. *Science.* 281(5381):1305–1308.

[36] Walczak, H., P.H. Krammer (2000) The CD95 (APO-1/Fas) and the TRAIL (APO-2L) apoptosis systems. *Exp Cell Res.* 256(1):58–66.

[37] Kuwana, T., L. Bouchier-Hayes, J.E. Chipuk (2005) BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. *Mol Cell.* 2005;17(4):525–535.

[38] Hassan, M., H. Watari, A. AbuAlmaaty, Y. Ohba, N. Sakuragi (2014) Apoptosis and Molecular Targeting Therapy in Cancer. *BioMed Res Int* (2014) Article ID 150845, 23 p., http://dx.doi.org/10.1155/2014/150845: 150845.

[39] Lavrik, I., A. Golks, P.H. Krammer (2005) Death receptor signaling. *J Cell Sci.* 118(2): 265–267.

[40] Su, M., Y. Mei, S. Sinha (2013) Role of the Crosstalk Between Autophagy and Apoptosis in Cancer. *J Oncology* (2013) Article ID 102735, 14 p., http://dx.doi.org/10.1155/2013/102735.

[41] Igney, F.H., P.H. Krammer (2002) Death and anti-death: tumour resistance to apoptosis. *Nat Rev Cancer*. 2(4):277–288.

[42] Wilson, J.W., M.C. Nostro, M. Balzi, P. Faraoni, F. Cianchi, A. Becciolini, C.S. Potten (2000) Bcl-w expression in colorectal adenocarcinoma. *Br J Cancer*. 82(1):178–85.

[43] Certo, M., V. Del Gaizo Moore, M. Nishino (2006) Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. *Cancer Cell*. 9:351–365.

[44] Kikuchi, Y., W.N. Dinjens, F.T. Bosman (1997) Proliferation and apoptosis in proliferative lesions of the colon and rectum. *Virchows Arch.* 431(2):111–7.

[45] Ilyas, M., X.P. Hao, K. Wilkinson, I.P. Tomlinson, A.M. Abbasi, A. Forbes, W.F. Bodmer, I.C. Talbot (1998) Loss of Bcl-2 expression correlates with tumour recurrence in colorectal cancer. *Gut*. 43:383–387.

[46] Zhang, Y.L., L.Q. Pang, Y. Wu, X.Y. Wang, C.Q. Wang, Y. Fan (2008) Significance of Bcl-xL in human colon carcinoma. *World J Gastroenterol.* 14(19):3069–3073.

[47] Huang, C.Y., L. Chia-Hui Yu (2015) Pathophysiological mechanisms of death resistance in colorectal carcinoma. *World J Gastroenterol.* 21(41):11777–11792.

[48] Wei, M.C., W.X. Zong, E.H. Cheng, T. Lindsten, V. Panoutsakopoulou, A.J. Ross, K.A. Roth, G.R. MacGregor, C.B. Thompson, S.J. Korsmeyer (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science.* 292(5517): 727–730.

[49] Kondo, S., Y. Shinomura, Y. Miyazak, T. Kiyohara, S. Tsutsui, S. Kitamura, Y. Nagasawa, M. Nakahara, S. Kanayama, Y. Matsuzawa (2000) Mutations of the bak gene in human gastric and colorectal cancers. *Cancer Res.* 60:4328–4330.

[50] Ionov, Y., H. Yamamoto, S. Krajewski, J.C. Reed, M. Perucho (2000) Mutational inactivation of the proapoptotic gene BAX confers selective advantage during tumor clonal evolution. *Proc Natl Acad Sci USA*. 97:10872–10877.

[51] Yagi, O.K., Y. Akiyama, T. Nomizu, T. Iwama, M. Endo, Y. Yuasa (1998) Proapoptotic gene BAX is frequently mutated in hereditary nonpolyposis colorectal cancers but not in adenomas. *Gastroenterology*. 114:268–274.

[52] De Angelis, P.M., T. Stokke, L. Thorstensen, R.A. Lothe, O.P. Clausenn (1998) Apoptosis and expression of Bax, Bcl-x, and Bcl-2 apoptotic regulatory proteins in colorectal carcinomas, and association with p53 genotype/phenotype. *Mol Pathol.* 51:254–261.

[53] Rampino, N., H. Yamamoto, Y. Ionov, Y. Li, H. Sawai, J.C. Reed, M. Perucho (1997) Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science.* 275:967–969.

[54] Jansson, A., X.F. Sun (2002) Bax expression decreases significantly from primary tumor to metastasis in colorectal cancer. *J Clin Oncol.* 20:811–816.

[55] Bai, L., W.G. Zhu (2006) p53: structure, function and therapeutic applications. *J Cancer Mol.* 2(4):141–153.

[56] Vikhanskaya, F., M.K. Lee, M. Mazzoletti, M. Broggini, K. Sabapathy (2007) Cancer-derived p53 mutants suppress p53-target gene expression—potential mechanism for gain of function of mutant p53. *Nucl Acids Res.* 35(6):2093–2104.

[57] Russo, A., V. Bazan, B. Iacopetta, D. Kerr, T. Soussi, N. Gebbia (2005) The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment. *J Clin Oncol.* 23:7518–7528.

[58] Katkoori, V.R., C. Shanmugam, X. Jia, S.P. Vitta, M. Sthanam, T. Callens, L. Messiaen, D. Chen, B. Zhang, H.L. Bumpers, T. Samuel, U. Manne (2012) Prognostic significance and gene expression profiles of p53 mutations in microsatellite-stable stage III colorectal adenocarcinomas. *Plos One.* 7:e30020.

[59] Huerta, S., E.J. Goulet, E.H. Livingston (2006) Colon cancer and apoptosis. *Am J Surg.* 191:517–526.

[60] Nyiraneza, C., A. Jouret-Mourin, A. Kartheuser, P. Camby, O. Plomteux, R. Detry, K. Dahan, C. Sempoux (2011) Distinctive patterns of p53 protein expression and microsatellite instability in human colorectal cancer. *Hum Pathol.* 42(12):1897–910.

[61] Edagawa, M., J. Kawauchi, M. Hirata, H. Goshima, M. Inoue, T. Okamoto, A. Murakami, Y. Maehara, S. Kitajima (2014) Role of activating transcription factor 3 (ATF3) in endoplasmic reticulum (ER) stress-induced sensitization of p53-deficient human colon cancer cells to tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL)-mediated apoptosis through upregulation of death receptor 5 (DR5) by zerumbone and celecoxib. *J Biol Chem.* 289:21544–21561.

[62] Wu, W.K., X.J. Wang, A.S. Cheng, M.X. Luo, S.S. Ng, K.F. To, F.K. Chan, C.H. Cho, J.J. Sung, J. Yu (2013) Dysregulation and crosstalk of cellular signaling pathways in colon carcinogenesis. *Crit Rev Oncol Hematol.* 86(3):251–277.

[63] Mita, A.C., M.M. Mita, S.T. Nawrocki, F.J. Giles (2008) Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. *Clin Cancer Res.* 14(16):5000–5005.

[64] Olsson, M., B. Zhivotovsky (2011) Caspases and cancer. *Cell Death Differ.* 18:1441–1449.

[65] Fink, S.L., B.T. Cookson (2005) Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect Immun*. 73(4):1907–1916.

[66] Devarajan, E., A.A. Sahin, J.S. Chen, R.R. Krishnamurthy, N. Aggarwal, A.M. Brun, A. Sapino, F. Zhang, D. Sharma, X.H. Yang, A.D. Tora, K. Mehta (2002) Downregulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance. *Oncogene.* 21(57):8843–8851.

[67] Shen, X.G., C. Wang, Y. Li, L. Wang, B. Zhou, B. Xu, X. Jiang, Z.G. Zhou, X.F. Sun (2010) Downregulation of caspase-9 is a frequent event in patients with stage II colorectal cancer and correlates with poor clinical outcome. *Colorectal Dis.* 12(12):1213–1218.

[68] Fong, P.C., W.C. Xue, H.Y.S. Ngan, P.M. Chiu, K.Y.K. Chan, G.S.W. Tsao, A.N.Y. Cheung (2006) Caspase activity is downregulated in choriocarcinoma: a cDNA array differential expression study. *J Clin Pathol.* 59(2):179–183.

[69] Fulda, S., K.M. Debatin (2006) Extrinsic versus intrinsic apoptosis pathways in anti-cancer chemotherapy. *Oncogene.* 25:4798–4811.

[70] Fulda, S., L. Galluzzi, G. Kroemer (2010) Targeting mitochondria for cancer therapy. *Nat Rev Drug Dis.* 9(6):447–464.

[71] Acharya, A., I. Das, D. Chandhok, T. Saha (2010) Redox regulation in cancer: a double-edged sword with therapeutic potential. *Oxid Med Cell Longev*. 3:23–34.

[72] Circu, M.L., T.Y. Aw (2010) Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med*. 48:749–762.

[73] Storz, P. (2005) Reactive oxygen species in tumor progression. *Front Biosci.* 10:1881–96.

[74] Afanas'ev, I. (2011) Reactive oxygen species signaling in cancer: comparison with aging. *Aging Dis*. 2(3):219–30.

[75] Dickinson, B.C., C.J. Chang (2011) Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat Chem Biol*. 7(8):504–11.

[76] Liou, G.Y., P. Storz (2010) Reactive oxygen species in cancer. *Free Radic Res.* 44(5):1–31.

[77] Waris, G, H. Ahsan (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog*. 5:14.

[78] Gupta, R.A., R.N. DuBois, M.C. Wallace (2002) New avenues for the prevention of colorectal cancer: targeting cyclo-oxygenase-2 activity. *Best Pract Res Clin Gastroenterol.* 16:945–56.

[79] Martindale, J.L., N.J. Holbrook (2002) Cellular response to oxidative stress: signaling for suicide and survival. *J Cell Physiol.* 192(1):1–15.

[80] Trachootham, D., J. Alexandre, P. Huang (2009) Targeting cancer cells by ROSmediated mechanisms: a radical therapeutic approach? *Natl Rev Drug Dis*. 8:579–591.

[81] Oberreuther-Moschner, D.L., G. Rechkemmer, B.L. PoolZobel (2005) Basal colon crypt cells are more sensitive than surface cells toward hydrogen peroxide, a factor of oxidative stress. *Toxicol Lett.* 159(3):212–218.

[82] Rainis, T., I. Maor, A. Lanir, S. Shnizer, A. Lavy (2007) Enhanced oxidative stress and leucocyte activation in neoplastic tissues of the colon. *Dig Dis Sci.* 52(2):526–530.

[83] Slattery, M.L., A. Lundgreen, B. Welbourn, R.K. Wolff, C. Corcoran (2012) Oxidative balance and colon and rectal cancer: interaction of lifestyle factors and genes. *Mutat Res.* 734(1–2):30–40.

[84] Colin, D.J., E. Limagne, K. Ragot, G. Lizard, F. Ghiringhelli, É. Solary, B. Chauffert, N. Latruffe, D. Delmas (2014) The role of reactive oxygen species and subsequent DNA-damage response in the emergence of resistance towards resveratrol in colon cancer models. *Cell Death Dis*. 5:1533–1546.

[85] Chiarugi, P, T. Fiaschi (2007) Redox signalling in anchorage-dependent cell growth. *Cell Signal*. 19(4):672–82.

[86] Guo, Z., S. Kozlov, M.F. Lavin, M.D. Person, T.T. Paull (2010) ATM activation by oxidative stress. *Science.* 330:517–521.

[87] Dhillon, A.S., S. Hagan, O. Rath, W. Kolch (2007) MAP kinase signalling pathways in cancer. *Oncogene.* 26(22):3279–3290.

[88] She, Q.B., A.M. Bode, W.Y. Ma, N.Y. Chen, Z.G. Dong (2001) Resveratrol-induced activation of p53 and apoptosis is mediated by extracellular-signal-regulated protein kinases and p38 kinase. *Cancer Res.* 61:1604–1610.

[89] Shih, A., F.B. Davis, H.Y. Lin, P.J. Davis (2002) Resveratrol induces apoptosis in thyroid cancer cell lines via a MAPK- and p53-dependent mechanism. *J Clin Endocrinol Metab.* 87:1223–1232.

[90] Aggarwal, B.B., S. Shishodia (2006) Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol*. 71:1397–1421.

[91] Kim, Y.H., D.H. Lee, J.H. Jeong, Z.S. Guo, Y.J. Lee (2008) Quercetin augments TRAIL induced apoptotic death: involvement of the ERK signal transduction pathway. *Biochem Pharmacol*. 75:1946–1958.

[92] Cagnol, S., J.C. Chambard (2010) ERK and cell death: mechanisms of ERK-induced cell death—apoptosis, autophagy and senescence. *FEBS J*. 277:2–21.

[93] Kong, E.H., Y.J. Kim, H.J. Cho, S.N. Yu, K.Y. Kim, J.H. Chang, S.C. Ahn (2008) Piplartine induces caspase-mediated apoptosis in PC-3 human prostate cancer cells. *Oncol Rep*. 20:785–792.

[94] Raj, L., T. Ide, A.U. Gurkar, M. Foley, M. Schenone, X. Li, N.J. Tolliday, T.R. Golub, S.A. Carr, A.F. Shamji, A.M. Stern, A. Mandinova, S.L. Schreiber, S.W. Lee (2011) Selective

killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature.* 475:231–234.

[95] Cadenas, E. (2004) Mitochondrial free radical production and cell signaling. *Mol Aspects Med*. 25(1–2):17–26.

[96] Storz, P. (2007) Mitochondrial ROS—radical detoxification, mediated by protein kinase D. *Trends Cell Biol.* 17(1):13–8.

[97] You, H., K. Yamamoto, T.W. Mak (2006) Regulation of transactivation-independent proapoptotic activity of p53 by FOXO3a. *Proc Natl Acad Sci USA*. 103(24):9051–6.

[98] Janssen-Heininger, Y.M., M.E. Poynter, P.A. Baeuerle (2000) Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. *Free Radic Biol Med.* 28(9):1317–27.

[99] Chen, G.G., J.F.Y. Lee, S.H. Wang, U.P.F. Chan, C Ip Ping, W.Y. Lau (2002) Apoptosis induced by activation of peroxisome-proliferator activated receptor-gamma is associated with Bcl-2 and Nf-κB in human colon cancer. *Life Sci.* 70(22):2631–2646.

[100] Santandreu, F.M., A. Valle, J. Oliver, P. Roca (2011) Resveratrol potentiates the cytotoxic oxidative stress induced by chemotherapy in human colon cancer cells. *Cell Physiol Biochem*. 28(2):219–28.

[101] Radhakrishnan, S., L. Reddivari, R. Sclafani, U.N. Das, J. Vanamala (2011) Resveratrol potentiates grape seed extract induced human colon cancer cell apoptosis. *Front Biosci.* 3:1509–1523.

[102] Zhang, C., G. Lin, W. Wan, X. Li, B. Zeng, B. Yang, C. Huang (2012) Resveratrol, a polyphenol phytoalexin, protects cardiomyocytes against anoxia/reoxygenation injury via the TLR4/NF-κB signaling pathway. *Int J Mol Med*. 29(4):557–563.

[103] Connors, T.A., R. Duncan, R.J. Knox (1995) The chemotherapy of colon cancer. *Eur J Cancer.* 31(7–8):1373–1378.

[104] Chan, A.T., E.L. Giovannucci (2010) Primary prevention of colorectal cancer. *Gastroenterology*. 138(6):2029–2043.

[105] Datsis, A., A. Tsoga, V. Langouretos (2010) The role of functional foods in the prevention of colorectal cancer. *Hell J Surg*. 82(4):224–232.

[106] Boursi, B., N. Arber (2007) Current and future clinical strategies in colon cancer prevention and the emerging role of chemoprevention. *Curr Pharm Des.* 13(22):2274–2282.

[107] Derry, M.M., R. Komal, A. Chapla, A. Rajesh (2013) Identifying molecular targets of lifestyle modifications in colon cancer prevention. *Front Oncol.* 3:119.

[108] Farlex Partner Medical Dictionary. (2012) Retrieved January 26, 2016 from http://medicaldictionary.thefreedictionary.com/chemoprevention

[109] Castillo-Pichardo, L., S.F. Dharmawardhane (2012) Grape polyphenols inhibit Akt/mammalian target of rapamycin signaling and potentiate the effects of gefitinib in breast cancer. *Nutr Cancer.* 64(7):1058–1069.

[110] Mahyar-Roemer, M., H. Köhler, K. Roemer (2002) Role of Bax in resveratrol-induced apoptosis of colorectal carcinoma cells. *BMC Cancer*. 2:27.

[111] Cal, C., H. Garban, A. Jazirehi, C. Yeh, Y. Mizutani, B. Bonavida (2003) Resveratrol and cancer: chemoprevention, apoptosis, and chemoimmunosensitizing activities. *Curr Med Chem Anti-Cancer Agents*. 3:77–93.

[112] Nicastro, H.L., E.B. Trujillo, J.A. Milner (2012) Nutrigenomics and cancer prevention. *Curr Nutr Rep*. 1(1):37–43.

[113] Banerjee, S., Y. Li, Z. Wang, F.H. Sarkar (2008) Multi-targeted therapy of cancer by genistein. *Cancer Lett*. 269(2):226–242.

[114] Nakamura, Y., S. Yogosawa, Y. Izutani, H. Watanabe, E. Otsuji, T. Sakai (2009) A combination of indole-3-carbinol and genistein synergistically induces apoptosis in human colon cancer HT-29 cells by inhibiting AKT phosphorylation and progression of autophagy. *Mol Cancer*. 8:100–115.

[115] Sanchez-Gonzalez, P.D., F. Lopez-Hernandez, F.P. Barriocanal, A.I. Morales, J.M.L. Novoa (2011) Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. *Nephrol Dial Transplant.* 26:3484–3495.

[116] Shan, B.E., M.X. Wang, R.Q. Li (2009) Quercetin inhibit human SW480 colon cancer growth in association with inhibition of cyclin D1 and survivin expression through Wnt/beta-catenin signaling pathway. *Cancer Investig.* 27(6):604–12.

[117] Kawamori, T., R. Lubet, V.E. Steele, G.J. Kelloff, R.B. Kaskey, C.V. Rao, B.S. Reddy (1999) Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer Res.* 59:597–601.

[118] Johnson, I.T., E.K. Lund (2007) Nutrition, obesity and colorectal cancer. *Aliment Pharmacol Ther*. 26(2):161–181.

[119] Shao, H., K. Jing, E. Mahmoud, H. Huang, X. Fang, C. Yu (2013) Apigenin sensitizes colon cancer cells to antitumor activity of ABT-263. *Mol Cancer Ther*. 12(12):2640–50.

[120] Singh, B.N., S. Shankar, R.K. Srivastava (2011) Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. *Biochem Pharmacol*. 82(12):1807–1821.

[121] Grabacka, M.M., M. Gawin, M. Pierzchalska (2014) Phytochemical modulators of mitochondria: the search for chemopreventive agents and supportive therapeutics. *Pharmaceuticals (Basel)*. 7(9):913–42.

[122] Zeng, H., O.N. Trujillo, M.P. Moyer, J.H. Botnen (2011) Prolonged sulforaphane treatment activates survival signaling in nontumorigenic NCM460 colon cells but apoptotic signaling in tumorigenic HCT116 colon cells. *Nutr Cancer*. 63(2):248–55.

[123] Tang, F.Y., C.J. Shih, L.H. Cheng, H.J. Ho, H.J. Chen (2008) Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. *Mol Nutr Food Res.* 52(6):646–54.

[124] Lin, M.C., F.Y. Wang, Y.H. Kuo, F.Y. Tang (2011) Cancer chemopreventive effects of lycopene: suppression of MMP-7 expression and cell invasion in human colon cancer cells. *J Agric Food Chem.* 59(20):11304–18.

[125] Bonnesen, C., I.M. Eggleston, J.D. Hayes (2001) Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. *Cancer Res.* 61(16):6120–30.

[126] Zheng, Q., Y. Hirose, N. Yoshimi, A. Murakami, K. Koshimizu, H. Ohigashi, K. Sakata, Y. Matsumoto, Y. Sayama, H. Mori (2002) Further investigation of the modifying effect of various chemopreventive agents on apoptosis and cell proliferation in human colon cancer cells. *J Cancer Res Clin Oncol*. 128(10):539–46.

[127] Kaur, M., B. Velmurugan, A. Tyagi, C. Agarwal, R.P. Singh, R. Agarwal (2010) Silibinin suppresses growth of human colorectal carcinoma SW480 cells in culture and xenograft through down-regulation of beta-catenin-dependent signaling. *Neoplasia.* 12(5):415–424.

[128] Kauntz, H., S. Bousserouel, F. Gosse, J. Marescaux, F. Raul (2012) Silibinin, a natural flavonoid, modulates the early expression of chemoprevention biomarkers in a preclinical model of colon carcinogenesis. *Int J Oncol*. 41(3):849–54.

[129] Lee, E.R., Y.J. Kang, H.J. Kim, H.Y. Choi, G.H. Kang, J.H. Kim, B.W. Kim, H.S. Jeong, Y.S. Park, S.G. Cho (2008) Regulation of apoptosis by modified naringenin derivatives in human colorectal carcinoma RKO cells. *J Cell Biochem.* 104(1):259–73.

[130] Akpinar-Bayizit, A., T. Ozcan, L. Yilmaz-Ersan (2012) The therapeutic potential of pomegranate and its products for prevention of cancer. In "*Cancer Prevention—From Mechanisms to Translational Benefits*", 331–373, InTech, Croatia, *Alexandros Georgakilas Ed.*, ISBN 978-953-51-0547-3.

[131] Adhami, V.M., N. Khan, H. Mukhtar (2009) Cancer chemoprevention by pomegranate: laboratory and clinical evidence. *Nutr Cancer.* 61(6):811–815.

[132] Jaganathan, S.K., M.V. Vellayappan, G. Narasimhan, E. Supriyanto (2014) Role of pomegranate and citrus fruit juices in colon cancer prevention. *World J Gastroenterol*. 20(16):4618–4625.

[133] Temraz, S., D. Mukherji, A. Shamseddine (2013) Potential targets for colorectal cancer prevention. *Int J Mol Sci.* 14:17279–17303.

[134] Rupnarain, C., Z. Dlamini, S. Naicker, K. Bhoola (2005) Colon cancer: genomics and apoptotic events. *Biol Chem.* 385(6):449–464.

[135] Galluzzi, L., O. Kepp, C. Trojel-Hansen, G. Kroemer (2012) Non-apoptotic functions of apoptosis-regulatory proteins. *EMBO Rep*. 13:322–330.

[136] Velmurugan, B., S.C. Gangar, M. Kaur, A. Tyagi, G. Deep, R. Agarwal (2010) Silibinin exerts sustained growth suppressive effect against human colon carcinoma SW480 xenograft by targeting multiple signaling molecules. *Pharm Res.* 27(10):2085–2097.

[137] Panaro, M.A., V. Carofiglio, A. Acquafredda, P. Cavallo, A. Cianciulli (2012) Anti-inflammatory effects of resveratrol occur via inhibition of lipopolysaccharide-induced NF-κB activation in Caco-2 and SW480 human colon cancer cells. *Br J Nutr.* 108:1623–1632.

[138] Kauntz, H., S. Bousserouel, F. Gosse, F. Raul (2012) The flavonolignan silibinin potentiates TRAIL-induced apoptosis in human colon adenocarcinoma and in derived TRAIL-resistant metastatic cells. *Apoptosis.* 17(8):797–809.

[139] Raina, K., C. Agarwal, R. Agarwal (2013) Effect of silibinin in human colorectal cancer cells: targeting the activation of NF-κB signaling. *Mol Carcinog.* 52:195–206.

[140] Hoh, C., D. Boocock, T. Marczylo, R. Singh, D.P. Berry, A.R. Dennison, A.J. Gescher (2006) Pilot study of oral silibinin, a putative chemopreventive agent, in colorectal cancer patients: Silibinin levels in plasma, colorectum, and liver and their pharmacodynamic consequences. *Clin Cancer Res.* 12(9):2944–2950.

[141] Yogosawa, S., Y. Yamada, S. Yasuda, Q. Sun, K. Takizawa, T. Sakai (2012) Dehydrozingerone, a structural analogue of curcumin, induces cell-cycle arrest at the G2/M phase and accumulates intracellular ROS in HT-29 human colon cancer cells. *J Nat Prod*. 75(12):2088–2093.

[142] Tu, S.P., H. Jin, J.D. Shi, L.M. Zhu, Y. Suo, G. Lu, A. Liu, T.C. Wang, C.S. Yang (2012) Curcumin induces the differentiation of myeloid-derived suppressor cells and inhibits their interaction with cancer cells and related tumor growth. *Cancer Prev Res.* 5(2):205–215.

[143] Camacho-Barquero, L., I. Villegas, J.M. Sánchez-Calvo, E. Talero, S. Sánchez-Fidalgo, V. Motilva, C. Alarcón de la Lastra (2007) Curcumin, a Curcuma longa constituent, acts on MAPK p38 pathway modulating COX-2 and iNOS expression in chronic experimental colitis. *Int Immunopharmacol.* 7(3):333–342.

[144] Chen, C., Y. Liu, Y. Chen, J. Xu (2011) C086, a novel analog of curcumin, induces growth inhibition and down-regulation of NFκB in colon cancer cells and xenograft tumors. C086, a novel analog of curcumin, induces growth inhibition and down-regulation of NFκB in colon cancer cells and xenograft tumors. *Cancer Biol Ther.* 12(9):797–807.

[145] Patel, B.B., D. Gupta, A.A. Elliott, V. Sengupta, Y. Yu, A.P.N. Majumdar (2010) Curcumin targets FOLFOX-surviving colon cancer cells via inhibition of EGFRs and IGF-1R. *Anticancer Res.* 30(2):319–325.

[146] He, Z.Y., C.B. Shi, H. Wen, F.L. Li, B.L. Wang, J. Wang (2011) Upregulation of p53 expression in patients with colorectal cancer by administration of curcumin. *Cancer Investig.* 29:208–213.

[147] Li, Y.H., Y.B. Niu, Y. Sun, F. Zhang, C.X. Liu, L. Fan, Q.B. Mei (2015) Role of phytochemicals in colorectal cancer prevention. *World J Gastroenterol.* 21(31):9262–9272.

[148] Khan, N., H. Mukhtar (2008) Multitargeted therapy of cancer by green tea polyphenols. *Cancer Lett.* 269(2):269–280.

[149] Larsen, C.A., R.H. Dashwood (2010) (-)-Epigallocatechin-3-gallate inhibits Met signaling, proliferation, and invasiveness in human colon cancer cells. *Arch Biochem Biophys.* 501(1):52–57.

[150] Thakur, V.S., A.R. Ruhul Amin, R.K. Paul, K. Gupta, K. Hastak, M.K. Agarwal, M.W. Jackson, D.N. Wald, H. Mukhtar, M.L. Agarwal (2010) P53-Dependent p21-mediated growth arrest pre-empts and protects HCT116 cells from PUMA-mediated apoptosis induced by EGCG. *Cancer Lett.* 296(2):225–232.

[151] Steinmann, J., J. Buer, T. Pietschmann, E. Steinmann (2013) Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. *Br J Pharmacol.* 168(5): 1059–1073.

[152] Yang, C.S., H. Wang, G.X. Li, Z. Yang, F. Guan, H. Jin (2011) Cancer prevention by tea: evidence from laboratory studies. *Pharmacol Res.* 64(2):113–122.

[153] Yang, H., K. Landis-Piwowar, T.H. Chan, Q.P. Dou (2011) Green tea polyphenols as proteasome inhibitors: implication in chemoprevention. *Curr Cancer Drug Targets.* 11(3):296–306.

[154] Carrasco-Pozo, C., M.L. Mizgier, H. Speisky, M. Gotteland (2012) Differential protective effects of quercetin, resveratrol, rutin and epigallocatechin gallate against mitochondrial dysfunction induced by indomethacin in Caco-2 cells. *Chemico-Biol. Interact.* 195:199–205.

[155] Min, K., T.K. Kwon (2014) Anticancer effects and molecular mechanisms of epigallocatechin-3-gallate. *Integr Med Res*. 3(1):16–24.

[156] Adachi, S., T. Nagao, H.I. Ingolfsson F.R. Maxfield, O.S. Andersen, L. Kopelovich, I.B. Weinstein (2007) The inhibitory effect of (-)-epigallocatechin gallate on activation of the epidermal growth factor receptor is associated with altered lipid order in HT29 colon cancer cells. *Cancer Res.* 67(13):6493–6501.

[157] Park, S.Y., Y.J. Jeong, S.H. Kim, J.Y. Jung, W.J. Kim (2013) Epigallocatechin gallate protects against nitric oxide-induced apoptosis via scavenging ROS and modulating the Bcl-2 family in human dental pulp cells. *J Toxicol Sci.* 38(3):371–8.

[158] Adams, L.S., N.P. Seeram, B.B. Aggarwal, Y. Takada, D. Sand, D. Heber (2006) Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. *J Agric Food.* 54(3):980–985.

[159] Kasimsetty, S.G., D. Blalonska, M.K. Reddy, G. Ma, S.I. Khan, D. Ferreira (2010) Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins. *J Agric Food Chem.* 58:2180–2187.

[160] Larrosa, M., F.A. Tomás-Barberán, J.C. Espín (2006) The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. *J Nutr Biochem.* 17:611–625.

[161] Patil, J.R., G.K. Jayaprakasha, K.N. Chidambara Murthy, S.E. Tichy, M.B. Chetti, B.S. Patil (2009) Apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles of Citrus aurantifolia. *Food Chem.* 114(4):1351–1358.

[162] Pan, M.H., W.J. Chen, S.Y. Lin-Shiau, C.T. Ho, J.K. Lin (2002) Tangeretin induces cell-cycle G1 arrest through inhibiting cyclin-dependent kinases 2 and 4 activities as well as elevating Cdk inhibitors p21 and p27 in human colorectal carcinoma cells. *Carcinogenesis.* 23:1677–1684.

[163] Totta, P., F. Acconcia, S. Leone, I. Cardillo, M. Marino (2004) Mechanisms of naringenin-induced apoptotic cascade in cancer cells: involvement of estrogen receptor alpha and beta signalling. *IUBMB Life.* 56(8):491–9.

[164] Fridrich, D., N. Teller, M. Esselen, G. Pahlke, D. Marko (2008) Comparison of delphinidin, quercetin and (-)-epigallocatechin-3-gallate as inhibitors of the EGFR and the ErbB2 receptor phosphorylation. *Mol Nutr Food Res.* 52:815–822.

[165] Li, M., Z. Zhang, D.L. Hill, X. Chen, H. Wang, R. Zhang (2005) Genistein, a dietary isoflavone, down-regulates the mdm2 oncogene at both transcriptional and posttranslational levels. *Cancer Res*. 65(18):8200–8.

[166] Liu, B., Z. Zhou, W. Zhou, J. Liu, Q. Zhang, J. Xia, J. Liu, N. Chen, M. Li, R. Zhu (2014) Resveratrol inhibits proliferation in human colorectal carcinoma cells by inducing G1/S-phase cell cycle arrest and apoptosis through caspase/cyclin-CDK pathways. *Mol Med Rep*. 10:1697–1702.

[167] Fouad, M.A., A.M. Agha, M.M. Merzabani, S.A. Shouman (2013) Resveratrol inhibits proliferation, angiogenesis and induces apoptosis in colon cancer cells: calorie restriction is the force to the cytotoxicity. *Hum Exp Toxicol*. 32:1067–1080.

[168] Turktekin, M., E. Konac, H.I. Onen, E. Alp, A. Yilmaz, S. Menevse (2011) Evaluation of the effects of the flavonoid apigenin on apoptotic pathway gene expression on the colon cancer cell line (HT29*). J Med Food*. 14(10):1107–17.

[169] Cho, H., H. Jung, H. Lee, H.C. Yi, H.K. Kwak, K.T. Hwang (2015) Chemopreventive activity of ellagitannins and their derivatives from black raspberry seeds on HT-29 colon cancer cells. *Food Funct.* 6(5):1675–83.

[170] Umesalma, S., P. Nagendraprabhu, G. Sudhandiran (2015) Ellagic acid inhibits proliferation and induced apoptosis via the Akt signaling pathway in HCT-15 colon adenocarcinoma cells. *Mol Cell Biochem.* 399(1–2):303–13.

[171] Huang, R.F., Y.J. Wei, B.S. Inbaraj, B.H. Chen (2015) Inhibition of colon cancer cell growth by nanoemulsion carrying gold nanoparticles and lycopene. *Int J Nanomed.* 10:2823–46.

[172] Trejo-Solís, C., J. Pedraza-Chaverrí, M. Torres-Ramos, D. Jiménez-Farfán, A. Cruz Salgado, N. Serrano-García, L. Osorio-Rico, J. Sotelo (2013) Multiple molecular and cellular mechanisms of action of lycopene in cancer inhibition. *Evid Based Complementary and Alternative Medicine* (2013) Article ID 705121, 17 p., http://dx.doi.org/10.1155/2013/705121.

[173] Stefanska, B., H. Karlic, F. Varga, K. Fabianowska-Majewska, A.G. Haslberger (2012) Epigenetic mechanisms in anti-cancer actions of bioactive food components—the implications in cancer prevention. *Br J Pharmacol.* 167:279–297.

[174] Choi, K., M.G. Jung, Y.H. Lee, J.C. Yoon, S.H. Kwon, H.B. Kang, M.J. Kim, J.H. Cha, Y.J. Kim, W.J. Jun, J.M. Lee, H.G. Yoon (2009) Epigallocatechin-3-gallate, a histone acetyltransferase inhibitor, inhibits EBV-induced B lymphocyte transformation via suppression of RelA acetylation. *Cancer Res*. 69(2):583–92.

[175] Eberhart, C.E., R.J. Coffey, A. Radhika, F.M. Giardiello, S. Ferrenbach, R.N. DuBois (1994) Up-regulation of cyclooxygenase-2 gene-expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology.* 107:1183–8.

[176] Saldanha, S.N., R. Kala, T.O. Tollefsbol (2014) Molecular mechanisms for inhibition of colon cancer cells by combined epigenetic-modulating epigallocatechin gallate and sodium butyrate. *Exp Cell Res.* 324(1):40–53.

[177] Ho, E., J.D. Clarke, R.H. Dashwood (2009) Dietary sulforaphane, a histone deacetylase inhibitor for cancer prevention. *J Nutr.* 139:2393–2396.

[178] Myzak, M.C., W.M. Dashwood, G.A. Orner, E. Ho, R.H. Dashwood (2006) Sulforaphane inhibits histone deacetylase in vivo and suppresses tumorigenesis in Apc-minus mice. *FASEB J.* 20(3):506–508.

[179] Traka, M., A. Gasper, J. Smith, C. Hawkey, Y. Bao, R. Mithen (2005) Transcriptome analysis of human colon Caco-2 cells exposed to sulforaphane. *J Nutr.* 135:1865–1872.

[180] Slaby, O., M. Sachlova, V. Brezkova, R. Hezova, A. Kovarikova, S. Bischofová, S. Sevcikova, J. Bienertova-Vasku, A. Vasku, M. Svoboda, R. Vyzula (2013) Identification of microRNAs regulated by isothiocyanates and association of polymorphisms inside their target sites with risk of sporadic colorectal cancer. *Nutr Cancer.* 65:247–254.

[181] Kaeberlein, M., T. McDonagh, B. Heltweg, J. Hixon, E.A. Westman, S.D. Caldwell, A. Napper, R. Curtis, P.S. DiStefano, S. Fields, A. Bedalov, B.K. Kennedy (2005) Substrate-specific activation of sirtuins by resveratrol. *J Biol Chem*. 280(17):17038–45.

[182] Tili, E, J.J. Michaille, H. Alder, S. Volinia, D. Delmas, N. Latruffe, C.M. Croce (2010) Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGF-beta signaling pathway in sw480 cells. *Biochem Pharmacol*. 80:2057–2065.

[183] Kumazaki, M., S. Noguchi, Y. Yasui, J. Iwasaki, H. Shinohara, N. Yamada, et al. (2013) Anti-cancer effects of naturally occurring compounds through modulation of signal transduction and miRNA expression in human colon cancer cells. *J Nutr Biochem*. 24:1849–1858.

[184] Del Follo-Martinez, A., N. Banerjee, X. Li, S. Safe, S. Mertens-Talcott (2013) Resveratrol and quercetin in combination have anticancer activity in colon cancer cells and repress oncogenic microRNA-27a. *Nutr Cancer.* 65:494–504.

[185] Knop, C., J. Putnik, M. Scheuermann, M. Schmitz (2010) Cutting Edge Technologies: Cell Analysis/2010, B. Ziebolz, ed., Springer Medizin, Springer Verlag, Heidelberg, Germany, 4–13, 58–68, 137–141.

[186] Hotnog, D., M. Mihaila, A. Botezatu, G.G. Matei, C. Hotnog, G. Anton, M. Bostan, L.I. Brasoveanu (2013) Genistein potentiates the apoptotic effect of 5-fluorouracyl in colon cancer cell lines. *Rom Biotechnol Lett.* 18(6):8751–8760.

[187] Hotnog, D., M. Mihaila, I.V. Iancu, G.G. Matei, C. Hotnog, G. Anton, M. Bostan, L.I. Brasoveanu (2013) Resveratrol modulates apoptosis in 5-fluorouracyl treated colon cancer cell lines. *Roum Arch Microbiol Immunol.* 72(4):255–264.

[188] Ivanov, K., N. Kolev, I. Shterev, A. Tonev, V. Ignatov, V. Bojkov, T. Kirilova (2014) Adjuvant treatment in colorectal cancer. *"Colorectal Cancer—Surgery, Diagnostics and Treatment"*, 305–328, InTech, Croatia, *Jim S. Khan Ed.*, ISBN 978-953-51-1231-0.

[189] Nita, M.E., H. Nagawa, O. Tominaga, N. Tsuno, S. Fujii, S. Sasaki, C.G. Fu, T. Takenoue, T. Tsuruo, T. Muto (1998) 5-fluorouracil induces apoptosis in human colon cancer cell lines with modulation of Bcl-2 family proteins. *Br J Cancer*. 78(8):986–992.

[190] Wolmark, N., H. Rockette, B. Fisher, D.L. Wickerham, C. Redmond, E.R. Fisher, J. Jones, E.P. Mamounas, L. Ore, N.J. Petrelli, et al. (1993) The benefit of leucovorin-modulated fluorouracil as postoperative adjuvant therapy for primary colon cancer: results from National Surgical Adjuvant Breast and Bowel Project protocol C-03. *J Clin Oncol*. 11(10): 1879–87.

[191] Lowe, S.W., H.E. Ruley, T. Jacks, D.E. Housman (1993) p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell.* 74(6):957–67.

[192] Piazza, G.A., A.K. Rahm, T.S. Finn, B.H. Fryer, H. Li, A.L. Stoumen, R. Pamukcu, D.J. Ahnen (1997) Apoptosis primarily accounts for the growth-inhibitory properties of

sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. *Cancer Res*. 57:2452–59.

[193] Violette, S., L. Poulain, E. Dussaulx, D. Pepin, A.M. Faussat, J. Chambaz, J.M. Lacorte, C. Staedel, T. Lesuffleur (2002) Resistance of colon cancer cells to long-term 5-fluorouracil exposure is correlated to the relative level of Bcl-2 and Bcl-XL in addition to Bax and p53 status. *Int J Cancer*. 98(4):498–504.

[194] Zhang, N., Y. Yin, S.J. Xu, W.S. Chen (2008) 5-Fluorouracil: mechanisms of resistance and reversal strategies. *Molecules.* 13(8):1551–1569.

[195] Tomé-Carneiro, J., M. Larrosa, A. González-Sarrías, F.A. Tomás-Barberán, M.T. García-Conesa, J.C. Espín (2013) Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. *Curr Pharm Des*. 19(34):6064–6093.

IntechOpen