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Dietary Anthocyanins: Impact on Colorectal Cancer and Mechanisms of Action

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1. Introduction

Colorectal cancer is the third most common malignancy in males and the second most common in females, with significant variations in the worldwide distribution, and remains among four leading causes of cancer deaths overall, shows global cancer statistics. The highest incident rates are found in economically developed countries, whereas the lowest rates are noted in Africa and South-Central (Jemal et al., 2011). However, striking increase in colorectal cancer incident trends is observed in areas historically at low risk, such as Spain and some Eastern European (the Czech Republic and Slovakia) and Eastern Asian countries (Japan). On the other hand, generally high incident rates over the past several decades are going down in the Unites States (Center et al., 2009). These recent "perturbations" in colorectal cancer trends probably result from a combination of risk factors, including obesity, sedentary lifestyle, increased prevalence of smoking, excessive alcohol consumption and "westernization" in dietary habits - a diet rich in red and processed meat and low intake of fruits and vegetables (Center et al., 2009; Chao et al., 2005; Jemal et al., 2011). Decreasing incident and mortality rates are mainly associated with colorectal cancer screening and improved treatment.

Prognosis of these patients depends on the stage of the cancer at diagnosis. As the AJCC (American Joint Committee on Cancer) stage increases from stage I to stage IV, the 5-year overall survival rates decrease dramatically, reaching 90% if the disease is detected early when still localized, though just 39% of colorectal cancers are found at this stage. Almost 25% of patients have a metastatic disease at diagnosis, with a 5-year survival of less than 10% (Goldberg et al., 2007). The primary treatment for colorectal cancer is surgical resection. More than two-thirds of patients undergo radical surgery, but 30-50% of patients who present with stage II or III tumors ultimately experience disease recurrence and distant metastases (Rodriguez-Moranta et al., 2006). Although a broader base of treatment options for metastatic colorectal cancer (mCRC) has evolved in recent years, 50 - 70% of mCRC

patients still cannot be subjected to radical resection of metastases and are candidates for palliative therapy only (Fornaro et al., 2010).
The drugs commonly used to treat mCRC are fluoropyrimidines (fluorouracil and capecitabine), irinotecan – a semisynthetic derivative of the natural alkaloid camptothecin, and oxaliplatin – a diaminocyclohexane platinum compound. More recently, two monoclonal antibodies have been approved for the treatment of advanced stages of colorectal cancer. Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), is broadly used in combination with fluoropyrimidine-based chemotherapy. Cetuximab, a chimeric monoclonal antibody against the epidermal growth factor receptor (EGFR), is used as monotherapy or together with irinotecan in irinotecan-resistant patients (Hess et al., 2010; Tol and Punt, 2010; Van Cutsem et al., 2009). These chemotherapy agents have significantly improved the prognoses and median overall survival. However, chemotherapy drug resistance occurs in nearly all patients and remains the most frequent cause of treatment failure (Candeil et al., 2004; Dallas et al., 2009), calling for finding novel agents capable of killing drug-resistant colorectal cancer cells.

2. Dietary compounds and cancer

2.1 Cancer prevention by diet

The possibility that fruit and vegetables might help to reduce the risk for various types of cancer raised great interest already in the 1970s. The first studies conducted to assess differences in cancer rates and diet between countries suggested that various dietary factors might have important effects on cancer risk (Armstrong and Doll, 1975; Bjelke, 1975).
In 1992, an epidemiological research with 156 studies on connection between the consumption of fruit and vegetables and cancer concluded that persons with a low fruit and vegetable intake face up to twice the risk of developing cancer compared to those with a high intake (Block et al., 1992). Several years later, a joint report by the World Cancer Research Fund together with the American Institute of Cancer Research found 'convincing' evidence that a high fruit and vegetable intake would reduce cancer of the colon and rectum (AIRC, 1997).
Unfortunately, 10 years later, an updated report released by the same organization and based on large prospective studies instead on case-control studies, downgraded these previous conclusions. The evidence that high intakes of fruit and/or vegetables decrease the risk for cancers of the mouth and pharynx, esophagus, stomach, colorectum and lung were judged 'probable' or 'limited- suggestive', so researchers did not confirm the earlier results (AIRC, 2007).
In a randomized dietary intervention trial, called The Polyp Prevention Trial, it was examined the effectiveness of a low-fat, high-fiber, high-fruit, and high-vegetable diet on adenoma recurrence. This study was the first to examine the association between flavonoid intake and colorectal adenoma recurrence. It was found that total flavonoid intake was not associated with colorectal adenoma recurrence, but they also detected during the trial a reduced risk of advanced adenoma recurrence with greater flavonol consumption (Bobe et al., 2008).
The European Prospective Investigation into Cancer and Nutrition in 2009 suggested that a high consumption of fruit and vegetables is associated with a reduced risk of CRC, especially of colon cancer but differs according to smoking status. An inverse association for

never and former smokers and a statistically non significant positive association for current smokers was observed (van Duijnhoven et al., 2009).
Key, on the other hand, by summarizing data recorded from large prospective studies or pooled analyses, recommended a diet which contains moderate amounts of fruit and vegetables in order to prevent deficiencies of any nutrients. Nevertheless, the available data suggest that, at least in relatively well-nourished populations, general increases in fruit and vegetable intake would not have much effect on cancer rates (Key, 2011).
Due, at least in part, to their anti-oxidant and anti-inflammatory activities, epidemiologic studies suggest that the consumption of anthocyanins lowers the risk of cardiovascular disease, diabetes, arthritis and cancer (Prior and Wu, 2006). Their activities are associated to their action at different molecular level: direct ability to scavenge reactive oxygen species (Wang and Jiao, 2000) or to induce phase II antioxidant and detoxifying enzymes (Shih et al., 2005; Shih et al., 2007).

2.2 Dietary compounds and tumor progression

Cancer cells differ from normal cells due to the following properties: unlimited replication potential, the absence of apoptosis, the absence of telomere shortening, angiogenesis and metastasis. Dietary compounds have been shown to affect molecular events involved in the initiation, promotion and progression of cancer, thereby inhibiting carcinogenesis. Furthermore, their inhibitory activity may ultimately suppress the final steps of carcinogenesis as well, namely angiogenesis and metastasis. The relationship between the frequency of consumption of vegetables and fruit and cancer risk is linked to a class of phytochemicals which flavonoids belong to.
The unlimited replication potential of cancer cells is a result of the inactivation of tumour suppressor genes. For instance, mutated p21 gene products are no longer able to bind to cyclin, thus cyclin-dependent kinase remains active and cell division becomes uncontrolled. Targeting these protein kinases using natural products has been seen as a promising approach in solving the cancer menace (Omura et al., 1995; Yasuzawa et al., 1986). Although research on protein kinases is still at an early stage, there is enough evidence that dietary compounds have useful potency and specificity against protein kinases of medicinal importance.
Resveratol has been shown by numerous reports to inhibit cell proliferation through the inhibition of cell-cycle progression at different stages (Aggarwal and Shishodia, 2006; Liang et al., 2003; Takagaki et al., 2005). Down-regulation of the cyclin D1/Cdk4 complex by resveratrol has been reported in colon cancer cell lines (Wolter et al., 2001) as well as resveratrol-induced G2 arrest through the inhibition of Cdk7 and Cdc2 kinases in colon carcinoma HT-29cells (Liang et al., 2003). Furthermore, an anthocyanin-rich extract caused cell cycle arrest and increased expression of the p27kip1 and p21WAF1/Cip1 genes and a 60% cancer cell growth inhibition (Malik et al., 2003).
Abnormalities in the ubiquitin-proteasome system have been implicated in many protein degradation disorders, including several types of cancer. This has made the proteasome an important target for anti-cancer drug discovery. Proteasome inhibitors can be categorized as synthetic and natural, where natural molecules are often more specific and potent than synthetic ones (D'Alessandro et al., 2009). Chen and colleagues showed that dietary flavonoids apigenin and quercetin inhibit proteasome, and this inhibition may contribute to their cancer-preventative effects (Chen et al., 2005). Furthermore, Kazi and colleagues also

showed that the tumor cell apoptosis-inducing ability of genistein (a soy flavonoid) is associated with its inhibition of proteosome activity (Kazi et al., 2003).

Apoptosis is triggered when normal cells are worn out. In cancer cells, the telomerase activity allows them to evade apoptosis by stabilizing and elongating telomeres through synthesis of de novo telomeric DNA (Naasani et al., 2003). Telomerase activity has been identified in most human tumors (Kim et al., 1994). A high telomerase activity has been linked to the degree of malignancy and likelihood of tumor progression (Fujiwara et al., 2000; Hiyama et al., 1995). Tea catechins, especially the degradation products of epigallocatechin gallate, epicatechin, quercetin, naringin and naringinin, have been found to inhibit telomerase activity (Naasani et al., 1998).

Angiogenesis, one of the hallmarks of cancer, vital to tumor growth and metastasis, is characterized by growth of new capillaries from preexisting vessels (Folkman, 1995). Cancer cells release vascular epithelial growth factor (VEGF), an angiogenic cytokine which stimulates blood vessel growth. Inhibition of VEGF has therefore become a primary target for anti-angiogenic strategies, and inhibitors directed against either VEGF or its receptor VEGFR-2, have been demonstrated to prevent vascularization and growth of a large number of experimental tumor types (Labrecque et al., 2005; Underiner et al., 2004). Ellagic acid (naturally occurring phenolic constituent in fruits and nuts) has been shown to inhibit VEGF-induced migration of endothelial cells (Labrecque et al., 2005). Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation (Lamy et al., 2002), and resveratol also inhibits vascular endothelial growth factor (VEGF)-induced angiogenic effects in the human umbilical vein endothelial cells through the abrogation of VEGF-mediated tyrosine phosphorylation of vascular endothelial (VE)-cadherin and its complex partner, b-catenin (Aggarwal and Shishodia, 2006; Lin et al., 2003). In addition, the flavonoid luteolin also inhibited both VEGF-induced survival and proliferation of the human umbilical vein endothelial cells (Bagchi et al., 2004). *In vitro* studies have shown that anthocyanin-rich berry extract formula exhibited a potent inhibitory effect on H_2O_2-induced VEGF expression. Anthocyanins suppress angiogenesis through the inhibition of H_2O_2- and tumor necrosis factor alpha (TNF-a)-induced VEGF expression, as well as through the inhibition of VEGF and VEGF receptor expression (Bagchi et al., 2004).

Metastasis occurs when cancer cells invade blood and lymphatic vessels and are transported to other cells and tissues in the body. Cancer cells produce proteinase enzymes that allow them to invade blood and lymphatic vessels. The matrix metalloproteinases (MMP) are a group of proteolytic enzymes that degrade the extracellular matrix (ECM) components (Nabeshima et al., 2002). MMP-2 and MMP-9 are two important MMPs in cell invasion as cancerous tissues and tumor cells have shown increased levels and activities of both MMP-2 and MMP-9 (Bernardo and Fridman, 2003).

Proanthocyanidins and flavonoids from cranberry and other *Vaccinium* berries have been shown to inhibit the expression of MMPs involved in remodeling the extracellular matrix (Pupa et al., 2002). Curcumin inhibits MMP-2, which is implicated in the formation of loose and primitive looking meshwork formed by aggressive cancers such as melanoma and prostate cancers (Aggarwal and Shishodia, 2006). Resveratrol has been found to cause a dose-dependent inhibition of PMA (Phorbol Myristate Aacetate)-induced increases in MMP-9 expression and activity and also the suppression of MMP-9 mRNA expression. Furthermore, Rose and colleagues found that phytochemicals from broccoli and rorripa have anti-invasive and anti-metalloproteinase activities (Rose et al., 2005). Purified ursolic

acid and hydroxycinnamate esters from cranberry fruit strongly inhibited expression of MMP-2 and MMP-9 activities at micromolar concentrations in fibrosarcoma cells (Cha et al., 1996). Anthocyanins from mulberry fruits and highbush blueberry (*V. angustifolium*) inhibited MMP-2 and MMP-9 activities (Huang et al., 2008; Matchett et al., 2005; Matchett et al., 2006). Delphinidin can inhibit invasion of human fibrosarcoma cells through down-regulation of MMP-2 and MMP-9, expression (Nagase et al., 1998). More recently, it has been demonstrated that black rice anthocyanins, cyanidin 3-glucoside and peonidin 3-glucoside, significantly reduce the expression of MMP-9 in diverse types of cancer cells (Chen et al., 2006). Furthermore, it was also demonstrated that the activities of MMP-2 and -9 were dose-dependently suppressed by anthocyanin treatment on HT-29 human colon cancer cells (Yun et al., 2010) and in HCT-116 human colon cancer cells through the activation of 38-MAPK and suppression of the PI3K/Akt pathway (Shin et al., 2011).

2.3 Flavonoids

Bioactive compounds that impart protective properties to plants against various pathological conditions are grouped under the name of phytochemicals. Active components of dietary phytochemicals which have been identified to protect against cancer include curcumin, resveratrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, diosgenin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, eugenol, isoeugenol, dithiolthiones, isothiocyanates, indole-3-carbinol, protease inhibitors, saponins, phytosterols, inositol hexaphosphate, Vitamin C, D-limonene, lutein, folic acid, beta carotene, selenium, Vitamin E, flavonoids, and dietary fiber (Aggarwal and Shishodia, 2006).

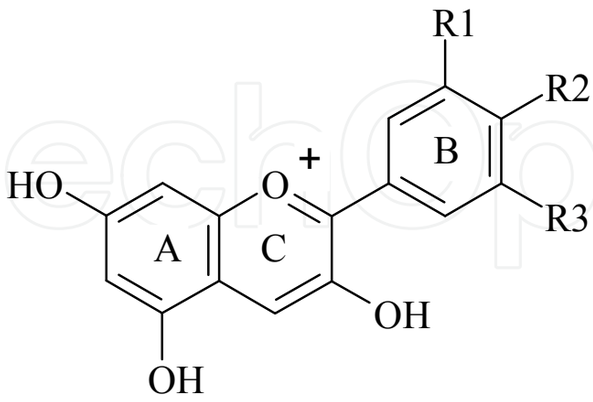
Flavonoids represent one of the largest groups of secondary metabolites whose name refers to a class of more than 6500 molecules based upon a 15-carbon skeleton (Harborne and Williams, 2000; Ververidis et al., 2007). They are divided into six major classes: flavanols, flavonones, flavones, isoflavones, flavonols and anthocyanins. Flavonoids are not synthesized in animal cells, thus their detection in animal tissues is indicative of plant ingestion (Mennen et al., 2008). Dietary flavonoids play an important role in cancer prevention and inhibition influencing various cellular processes, such as reactive oxygen species production and cell signal transduction pathways related to cellular proliferation, apoptosis, and angiogenesis (Yao et al., 2011).

Flavonoids compounds are the most studied anticarcinogens among phytochemicals. Anthocyanins, a particular class of this group of molecules, are the most abundant flavonoid constituents of fruits and vegetables (Wang and Stoner, 2008).

2.3.1 Anthocyanins: Chemistry

Anthocyanins (Greek anthos = flower and kyanos = blue) are water-soluble pigments in fruits and vegetables, responsible for red, blue and purple colors. In plant cells, they are present in vacuoles in the form of various sized granules. Their basic anthocyanidin aglycone structures consist of an aromatic ring A bonded to a heterocyclic ring C that contains oxygen, which is also bound by carbon-carbon bond to a third aromatic ring B (Figure 1). Anthocyanins normally occur in nature in glycoside forms. The sugar moiety is mainly attached to the C ring (in the 3-position) or to the A ring (in the 5, 7-position). Glucose, galactose, arabinose, rhamnose and xylose are the most common sugars bonded to the anthocyanidins. These glycosylated forms are known as anthocyanins. More than 500 different anthocyanins have been found, among which the most common is cyanidin

3-glucoside. The most common anthocyanidins (anthocyanins aglycones) found in nature are pelargonidin, peonidin, cyanidin, malvidin, petunidin and delphinidin (Figure 1) (Castañeda-Ovando et al., 2009; Manach et al., 2004; Szajdek and Borowska, 2008).



Anthocyanidin	R1	R2	R3
Pelargonidin	H	OH	H
Cyanidin	OH	OH	H
Delphinidin	OH	OH	OH
Peonidin	OCH_3	OH	H
Petunidin	OCH_3	OH	OH
Malvidin	OCH_3	OH	OCH_3

Fig. 1. Chemical structures of anthocyanidins (Prior and Wu, 2006).

2.3.2 Fate of anthocyanins in the gastro-intestinal tract

The lack of the knowledge of anthocyanin metabolism in the gastrointestinal tract has been studied by many authors (Aura, 2005; Hassimotto et al., 2008; He et al., 2009; McGhie and Walton, 2007; Vitaglione et al., 2007). The fate of anthocyanins in the gastrointestinal tract is summarized in Table 1.

Part of gastrointestinal tact	Anthocyanin fate
Mouth	Deglycosylation? (McGhie and Walton, 2007; Selma et al., 2009)
Stomach	Chemical stability (Hassimotto et al., 2008; McDougall et al., 2005) Absorption (Felgines et al., 2008; Passamonti et al., 2003b)
Small intestine	Deglycosylation, degradation, absorption
Large intestine	Deglycosylation, degradation (Talavera et al., 2004)

Table 1. Fate of anthocyanins through the gastrointestinal pathway

There are no data of the effect of saliva on anthocyanins but some publications suggest that flavonoid glycosides are hydrolyzed to corresponding aglycons (McGhie and Walton, 2007; Selma et al., 2009).

In the stomach, anthocyanins remain intact due to the low pH that shifts the molecules toward the most stabile flavylium cation. Anthocyanins absorption takes place in the stomach through active transport (including transport carriers such as bilitranslocase (Passamonti et al., 2003b) and sodium dependent glucose transporter (Felgines et al., 2008)) and continues in the small intestine (Talavera et al., 2004).

At the intestine neutral pH anthocyanins exist in equilibrium of four molecular forms (flavylium cation, quinoidal base, carbinol pseudobase and chalcone pseudobase) thus they can be easily exposed to degradation (McDougall et al., 2005). First studies showed degradation of anthocyanins from tart cherries to phenolic acids (Seeram et al., 2001). Later, their degradation was demonstrated by two steps. The first step is deglycosylation of anthocyanins to anthocyanidin aglycon while the second step is degradation of the formed aglycon to phenolic acid and aldehyde (Ávila et al., 2009; Fleschhut et al., 2006).

Deglycosylation is the cleavage of the glycosyl moiety from anthocyanins structure to form anthocyanidin aglycons.

These reactions could take place due to intestinal microflora (Aura et al., 2005b; Ávila et al., 2009; Fleschhut et al., 2006), under intestinal conditions at pH 7 (Fleschhut et al., 2006), or spontaneously in the presence of intestinal epithelial cells (Hassimotto et al., 2008; Kay et al., 2009).

Degradation of anthocyanidin aglycon, achieved spontaneously or by microflora (Ávila et al., 2009; Fleschhut et al., 2006; Forester and Waterhouse, 2008), represents the breakdown of its heterocycle and cleavage of the C-ring to form phenolic acid and aldehyde (Keppler and Humpf, 2005). Spontaneous degradation is a consequence of the neutral pH because anthocyanidin aglycones are observed in chalcone form which is rather unstable and can be easily degraded (Fleschhut et al., 2006; Keppler and Humpf, 2005). Data showed that major degradation products of anthocyanidin aglycons degraded to corresponding phenolic acids (Table 2), as well to some other less present products still unidentified (Ávila et al., 2009; Fleschhut et al., 2006; Forester and Waterhouse, 2008). Further phenolic acids can be transformed to the benzoic acids in the presence of intestinal bacteria by cleavage of the hydroxyl group in the 4-position (Aura et al., 2005a; Selma et al., 2009).

Anthocyanidin aglycon	Corresponding phenolic acid
Cyanidin	Protocatechuic acid
Delphinidin	Gallic acid
Pelargonidin	4-hydroxybenzoic acid
Malvidin	Syringic acid
Peonidin	Vanilic acid
Petunidin	3-*O*-methylgallic acid

Table 2. Degradation products of anthocyanidin aglycons

The fastest degraded were anthocyanidin aglycons, much faster than anthocyanin monoglycosides (Keppler and Humpf, 2005). As well anthocyanin degradation by intestinal microflora was much faster than spontaneous one (Forester and Waterhouse, 2008).

Anthocyanins that are not absorbed or degraded in the gastrointestinal tract can be excreted as intact forms. Unchanged anthocyanins were detected in human fecal samples 24 hours after blood orange juice consumption (Vitaglione et al., 2007), as well as in fecal samples collected from rats previously fattened by chokeberries, bilberries and grapes (He et al., 2005).

3. Citotoxicity/apoptosis of anthocyanins on colon cancer cells

As mentioned above, naturally occurring dietary substances, in particular, flavonoids, have gained increased attention as agents interfering with processes involved in cancer development and progression. Among them, anthocyanins might be of particular interest since their daily intake is remarkably high compared to other flavonoids - it is estimated to vary between 180 and 215 mg (Hou, 2003) whereas the intake of other flavonoids reaches only 20-25 mg/day. Numerous recent studies indicate that anthocyanins are able to inhibit the growth of embryonic fibroblasts and of different cancer cells derived from malignant human tissues, suggesting their possible role as chemopreventive agents. This brings in focus their possible importance for public health as dietary components with preventive impact on cancer as well as effective, cheap and safe anticancer supplements.

3.1 Cytotoxicity in colon and other cancer cell lines

There are few reports on the inhibitory effects of anthocyanins on colon cancer cell growth. Extracts of grapes, bilberries and chokeberries rich in anthocyanins have been shown to inhibit the growth of human malignant HT-29 colon cancer cells but did not affect the growth of non-malignant colon-derived cells (Zhao et al., 2004). Similar effect was observed in highly and low tumorigenic colon cancer cell lines, LoVo/Adr and LoVo. While delphinidin and cyanidin were cytotoxic and induced apoptosis in the former, they failed to demonstrate a similar effect in the latter (Cvorovic et al., 2010). Anthocyanins from tart cherries significantly reduced proliferation of human colon cancer cells HT29 and HCT-116 as well (Kang et al., 2003; Marko et al., 2004). An anthocyanins extract from Vaccinium uliginosum suppressed the growth of human colorectal cancer cells DLD-1 and COLO205 in a dose-dependent manner through the induction of apoptosis. It was hypothesized that the anticancer efficacy might be attributed to its high percentage of malvidin (Zu et al., 2010). On the other hand, the antiproliferative and the anti-cancer potential of several berry extracts containing different profiles of phenolic compounds (anthocyanins, flavonols, and ellagitannins) was studied in human colon cancer HT-29 cells. All the berry extracts studied decreased the proliferation and the number of HT-29 cells in the G0/G1 phase of the cell cycle. This correlated with their anthocyanin concentration supporting the fact that the inhibitory effect of berry extracts is based on the concentration rather than the composition of anthocyanins (Coates et al., 2007; Johnson et al., 2011; Wu et al., 2007).

Numerous studies reported antiproliferative activity of anthocyanins in human cancer cells derived from malignant tissues of various origins such as breast, lung, uterus, stomach, central nervous system, vulva, prostate (Lazze et al., 2004; Meiers et al., 2001; Olsson et al., 2004; Seeram et al., 2004; Zhang et al., 2005). Anthocyanins were potent and selective in inhibiting human promyelocytic leukemia cell proliferation as well (Feng et al., 2007; Hou et al., 2003; Katsube et al., 2003).

Animal studies have also reported anticarcinogenic properties of anthocyanins. In induced rat colon cancer cell models they significantly decreased total tumors as well as aberrant crypts (Hagiwara et al., 2001; Hagiwara et al., 2002; Harris et al., 2001; Lala et al., 2006; Magnusson et al., 2003). Cai and colleagues demonstrated that Red grape pomace extract (oenocyanin) interferes with adenoma development in the Apc^{Min} mouse by affecting tumor burden more prominently than tumor number. Oenocyanin efficacy was accompanied by the decreased adenoma cell proliferation and down-regulation of expression of the PI3 pathway component Akt, which supports cell proliferation (Cai et al., 2010). It was also

demonstrated in the same animal model that anthocyanin-rich tart cherry extract added to the drinking water was associated with fewer and smaller tumors in the cecum, but none of the tested treatments influenced the number of tumors in the small intestine or the number or burden of tumors in the colon. It was supposed, therefore, that lack of effect of anthocyanins on colonic tumor development may be a consequence of their metabolism by intestinal bacteria or their spontaneous degradation in the cecal and colonic environment (Bobe et al., 2006; Kang et al., 2003). Moreover, it was shown in Apc^{Min} mice that dietary consumption of anthocyanins in the form of either a mixture (Mirtoselect) or as a pure compound (cyanidin-3-glucoside) interferes with small intestinal adenoma development in a dose-dependent fashion. Authors remarked the presence of measurable levels of anthocyanins in the target organ and in the urine, and in concentrations near or below the detection limit in the systemic circulation. Unfortunately, the dietary dose, at which either agent was significantly efficacious when extrapolated by dose/ surface area comparison, suggested that equivalent for humans can be found in 740 g bilberries, that is a hefty dose. In terms of absolute dose of agent, cyanidin-3-glucoside was less efficacious than the Mirtoselect mixture. Furthermore, authors suggested that different results obtained, in comparison with other studies (Kang et al., 2003) were possibly due in part to the higher dose of anthocyanins employed but also due to the different way of administration since anthocyanins tend to be unstable in aqueous solution at neutral pH (Cooke et al., 2006). Recently, bilberry [(BB),Vaccinium myrtillus], lingonberry (LB, Vaccinium vitis-idaea), and cloudberry (CB, Rubus chamaemorus), rich in anthocyanins, proanthocyanidins and ellagic acid respectively, proved to be chemopreventive as demonstrated by a significant reduction in the number of intestinal tumors in Min/1 mice. Concerning their different chemical composition, authors suggested that the effects seen, may rather be a result of a mixture of compounds acting in synergy than an effect of a single active substance. Moreover, since the cellular levels of b-catenin are increased at all stages of colon carcinogenesis, it was demonstrated that two of these berries, LB and CB, markedly inhibited the growth of the adenomas and accumulation of nuclear b-catenin and cyclin D1. Unfortunately, also in this study, the amount of berries in the diets was high and could not be easily reached in a human diet (Misikangas et al., 2007).

Concerning other tumor models, the incidence, multiplicity and final mass of mammary tumors were significantly reduced in rats that would receive grape juice containing 15 different anthocyanins (Singletary et al., 2003). Cyanidin-3-glucoside reduced the size of lung cancer xenografts and significantly inhibited metastasis in nude mice (Ding et al., 2006). Lyophilized black raspberries prevented the development of NMBA (N-nitrosomethylbenzylamine)-induced esophageal tumors (Stoner et al., 2007), just like anthocyanin-containing pomegranate extract delayed the onset and reduced the incidence of DMBA (7,12-dimethylbenzanthracene)-induced skin tumors in CD-1 mice.

Epidemiological studies in humans are, however, still scarce and contradictory. Biopsies of tumor and normal-appearing tissues in colon cancer patients consuming black raspberry powder daily during several weeks, showed reduced proliferation and increased apoptosis in cancerous but not in normal tissue. Antiangiogenic effect was also observed in these patients (Wang et al., 2007). A phase I pilot study in colorectal cancer patients demonstrated that treatment with black raspberries caused positive modulation of biomarkers of tumor development, including cell proliferation, apoptosis, angiogenesis and Wnt pathway in both colorectal adenocarcinomas and adjacent normal tissues (Wang et al., 2007). In a clinical pilot study twenty-five colorectal cancer patients, scheduled to undergo resection of primary

tumor or liver metastases, received different amount of mirtocyan. This is a standardized anthocyanin mixture extracted from bilberries administered daily for 7 days before surgery. In the immunohistochemical observations of colorectal tumors from all patients who had received mirtocyan, in comparison with the preintervention biopsy, the proliferation index, reflected by Ki-67 staining, was significantly decreased by 7%. The apoptotic index in colorectal cancer samples from all patients increased from 3.6% to 5.3% of epithelial cells. However, in the absence of a zero dose control group, authors couldn't determine if this increase could, at least, to some extent, be the consequence of inherent procedural differences in measurements. Nevertheless, the pharmacodynamic changes observed seemed to be more prominent in patients at a dose of anthocyanins, which elicited target tissue levels below the detection limit, than at higher one, which furnished detectable anthocyanin levels in colorectal tissue (Thomasset et al., 2009).

However, an Italian study aimed at investigating the relationship between anthocyanidins intake and risk for oral or pharyngeal cancer did not show any significant association (Rossi et al., 2007). There was no protective effect demonstrated on the development of prostate cancer either (Bosetti et al., 2006). Optimal tumor inhibition occurs when the berry anthocyanins are added to the diet before, during and after treatment with carcinogens, suggesting that consumption of berries throughout life may maximize their chemopreventive effectiveness in humans. The fact that berry diets show a variable effect on tumorigenesis suggests that the inhibitory components of berry extract are not completely absorbed and/or that molecules housed in berry extracts do not affect certain critical signaling pathways of carcinogenesis (Stoner, 2009). Although further proves are needed, these studies open a possibility for anthocyanins to be considered for use in cancer treatment in combination with other therapeutic methods.

3.2 How anthocyanins work – The mechanisms

Antimutagenic and anticarcinogenic activity of anthocyanins is generally ascribed to their antioxidant properties conveyed by their phenolic structure. The double bonds in the ring and the hydroxyl side chains confers them potent free-radical scavenging activities (the positively charged oxygen atom in their molecule makes them more generous hydrogen-donating antioxidants compared to other flavonoids), but also enables their metal chelation and protein binding properties (Kong et al., 2003). Apart from acting as direct free-radical scavengers, anthocyanins have been demonstrated to affect the activity of phase II enzymes well-known for their detoxifying and antioxidant properties and therefore important in cancer prevention. *In vivo* studies showed that the diet supplemented with freeze-dried blueberries or black raspberries, both rich in anthocyanins, led to increased glutathione S-transferase (GST) activity in rats (Boateng et al., 2007; Reen et al., 2006). On the other hand, intake of an anthocyanin-rich mixed berry juice reduced oxidative DNA damage in peripheral-blood mononuclear cells and significantly increased total glutathione (GSH) level and GSH status in whole blood in male healthy non-smoking probands (Weisel et al., 2006). All this speaks in favor of a multi-level antioxidant activity of anthocyanins.

However, numerous recent studies, on the anthocyanins role in tumor growth inhibition, point at their prooxidant properties. It has been shown that the apoptotic effect of anthocyanins in malignant cells could be result of their ability to induce ROS accumulation in these cells. Moreover, the apoptotic activity was directly correlated to the number of hydroxyl groups at the B-ring (Hou et al., 2003). Interestingly, ROS generation was observed in leukemia cells treated with cyanidin-3-rutinoside, but not in normal human peripheral-

blood mononuclear cells. Parallel with the accumulation of ROS, Feng and colleagues demonstrated the increase of peroxides, but not superoxides in these cells, suggesting the reaction with the glutathione antioxidant system as one of the possible mechanisms for this prooxidant activity, together with ROS-dependent activation of p38 and JNK (Feng et al., 2007). Similarly, both delphinidin and cyanidin, showed prooxidant activity and induced apoptotic changes and cytotoxic effect in metastatic colorectal drug-resistant cells (LoVo/ADR), but not in cells originating from primary tumor site, Caco-2 (Cvorovic et al., 2010). This "inconsistent" behavior of the anthocyanidins might be influenced by cellular energy metabolism changes associated with neoplastic transformation (Warburg, 1956b). Indeed, the rate of lactate production is significantly higher in highly tumorigenic LoVo/ADR than in low tumorigenic LoVo cells (Fanciulli et al., 2000), and, presumably, in CaCo-2 as well. And even a slight decrease of pH might favor protonation of anthocyanidins, a mechanism causing loss of their free-radical scavenging activity (Borkowski et al., 2005). However, it is not clear if anthocyanidins directly promote oxidative stress in LoVo/ADR cells. One of the possible mechanisms proposed in this study is the interference with the glutathione antioxidant system. Delphinidin and cyanidin inhibited glutathione reductase (GR) activity in LoVo/ADR cells and significantly depleted their intracellular glutathione levels, while failing to induce any similar effect in CaCo-2 cells. These studies give evidence that anthocyanins preferentially kill cancer cells with high malignant characteristics and resistant to conventional treatment regimens, which could set the basis for the development of new sensitizing agents in the treatment of metastatic disease.

4. Biochemical features accompanying cytotoxicity/apoptosis

4.1 Membrane transport of anthocyanins

All the metabolic actions exerted by anthocyanins imply their cellular bioavailability. Previous *in vivo* studies have reported that anthocyanins are absorbed in the stomach and small intestine (Passamonti et al., 2003a; Talavera et al., 2003; Talavera et al., 2004). Felgines and colleagues administered an oral dose of a radiolabelled cyanidin 3-O-glucoside, demonstrating that the major site of absorption in mice is the intestine with a minimal accumulation of the radioactivity in tissues out of the gastrointestinal tract (Felgines et al., 2010).

Intestinal barrier is impermeable to most flavonoid glucosides because, based on their molecular structure, anthocyanins and their aglycones cannot cross the cell membrane passively (Dreiseitel et al., 2009). Among the influx carriers, the hexose transporters SGLT1 and GLUT 5 are expressed on apical membrane of the intestinal epithelium. Different groups suggested that anthocyanins, based on their glycosides moiety, could be transported by glucose carrier SGLT1. However, the involvement of this protein it is not completely understood (Milane et al., 2007; Talavera et al., 2004; Wolffram et al., 2002).

Recently, it was also demonstrated that GLUT2, another glucose transporter, is expressed not only at the basolateral but also at apical membranes of intestinal cells. Faria and colleagues showed that kinetic parameters of ^{3}H-2-deoxy-D-glucose-uptake of GLUT2 are changed after acute treatment with anthocyanins, supporting a favorable use of anthocyanins in diabetic population. Interestingly they also observed an increased GLUT2 expression (not for SGLT1 or GLUT5) after a chronic exposure to anthocyanins speculating that this behavior could increase their own bioavailability (Faria et al., 2009).

Bilitranslocase (BTL) is an organic anions transporter specific for bilirubin, initially found in the membranes of hepatic sinusoidal cells, but present also at the gastric mucosa and in renal tubules (Baldini et al., 1986; Elias et al., 1990; Sottocasa et al., 1989). Some of its substrates are nicotinic acid, bromosulfophthalein, cibacron blue and some flavonoids (Passamonti et al., 2009; Passamonti et al., 2002). Among flavonoids family, 17 anthocyanins showed competitive inhibitory behavior to specific transport assay with delphinidin as the most active molecule (Passamonti et al., 2002). It was also demonstrated that the BTL is directly involved in the vasoactivity of flavonoids: vasorelaxation induced by both, cyanidin 3-glucoside and bilberry anthocyanins, was significantly decreased in aorta rings pre-treated with anti-BTL antibodies (Ziberna et al., 2011). Recent studies have revealed that bilitranslocase is also expressed at the intestinal epithelial level, in particular, at the apical domain. Caco-2 cells express BTL as well and the uptake of BSP into these cells is strongly inhibited by anti-bilitranslocase antibodies (Passamonti et al., 2009).
The results reported should be further implemented to clarify the involvement of the influx membrane transporters.
More data are available on flavonoids efflux transporters. Major interest on these proteins is linked to their involvement in cancer resistance. These proteins belong to the class of the ABC transporters (ATP-binding cassette), and their role in cancer cells is to prevent the accumulation of anticancer drugs. Some ABC transporters are MRP1 (multidrug resistance-associated protein 1, ABCC1) (Cole et al., 1992), MRP2 (ABCC2) and MRP3 (ABCC3) (Borst et al., 1999) as well as BCRP/MXR1 (ABCG2) (Doyle et al., 1998; Miyake et al., 1999) and Breast Cancer Resistance Protein BCRP (ABCG2) however, P-glycoprotein (ABCB1) is overexpressed to the highest level and plays the major role. It was shown that flavonoids interact with these transporters but their effects are often contradictory depending on the type of cancer cells (Di Pietro et al., 2002). Moreover, a different behavior depending on the molecular structure was also demonstrated. Dreiseitel and colleagues showed that, depending on the sugar moiety, some flavonoids can act as BCRP stimulators while others act as inhibitors (Dreiseitel et al., 2009; Morris and Zhang, 2006).
However, the significance of these flavonoid–efflux transporter interactions has not been unequivocally demonstrated since it is impossible to exclude the involvement of other drugs transporters and of intracellular metabolizing enzymes that modify the substrate disposition (Morris and Zhang, 2006).

4.2 Oxidative stress and apoptosis

Reactive oxygen species (ROS) and reactive nitrogen species (RONS) are a collective term that broadly describes O_2-derived free radicals such as superoxide anions ($O2^{\bullet -}$), hydroxyl radicals (HO•), peroxyl (RO_2•) and alkoxyl radicals (RO•), nitrogen monoxide (NO•), peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2•) as well as O_2-derived non-radical species such as hydrogen peroxide (H_2O_2) (Halliwell and Cross, 1994). Both reactive species are important mediators in the normal regulation of different physiological processes such as cellular proliferation or activation. On the other hand, the imbalance of cellular redox homeostasis is described at the base of many chronic diseases and is also involved in cancer development (Acharya et al., 2010).
Specific ROS such as H_2O_2 or superoxide have been implicated as crucial mediators of apoptotic cell death (Casado et al., 2002; Circu and Aw, 2010; Madeo et al., 1999). ROS tend to enhance survival or promote cell death by activating different factors such as members of

the mitogen-activated protein kinases (MAPKs), phosphatidylinositol-3-kinase (PI3K)/Akt pathway, phospholipase C-g1 (PLCg1) signaling, protein kinase C, p53 signaling, ataxia-telangiectasia-mutated (ATM) kinase, nuclear factor-kappaB (NF-kB) signaling, and Jak/Stat pathway. ROS modulate the apoptotic signaling pathway through the cellular redox status by activating key protein kinases (Chan et al., 2010; Noguchi et al., 2005). Pro-oxidants such as H_2O_2 or other stressors, could induce apoptosis (or programmed cell death) by activating the intrinsic or "mitochondrial" apoptosis pathway that results in the damage of this sub-cellular compartment and the pro-apoptotic factors release (Circu and Aw, 2010; Mates et al., 2008). ROS involved in apoptosis derive both from environmental pro-oxidants or from intracellular respiratory dysfunction since mitochondria are the main site of intracellular source of ROS production. It was reported that oxidative stress plays an important role in the molecular mechanism of colorectal cancer (Keshavarzian et al., 1992) Flavones in HT-29 colon cancer cells increase the uptake of pyruvate or lactate into mitochondria, which is followed by an increase in O_2^- production that finally leads to apoptosis (Wenzel et al., 2005).

The prooxidant activity of anthocyanins through the increase of intracellular ROS production has been clearly explained in several studies (Feng et al., 2007; Hou et al., 2005).

4.3 GSH role in apoptosis

Intracellular glutathione (GSH) is a major buffer of cellular redox status due to its active SH-group that has reducing nucleophilic properties (Meister, 1983; Meister, 1991; Meister and Anderson, 1983). It acts as reducing agent, antioxidant and free-radical scavenger against ROS generated during oxidative metabolism and/or oxidative stress (Donati et al., 1990; Hall, 1999a; Hall, 1999b; Sies, 1999) and is also involved in the metabolism of xenobiotics and some cellular molecules (Wu et al., 2004). Free glutathione is present mainly in its reduced form maintained by the action of glutathione reductase (GR), but chemical oxidation of GSH to GSSG can occur as a result of numerous enzyme-catalysed reactions that use GSH to reduce hydrogen peroxide or other peroxides to water or the corresponding alcohol (Diaz Vivancos et al., 2010). GSH is preferentially (85-90%) located in the cytosolic-nuclear compartments and only a small amount is present in mitochondria and endoplasmic reticulum (Hwang et al., 1992; Meredith and Reed, 1982). The free-radical and antioxidant action of GSH depends on its involvement in different enzymatic reactions as those catalyzed by glutathione peroxidases (GPxs) (Lei, 2002), glutathione-S-transferases (GSTs), formaldehyde dehydrogenase, maleylacetoacetate isomerase, and glyoxalase I (Arrigo, 1999; Dickinson and Forman, 2002; Dickinson et al., 2002; Hayes and McLellan, 1999). Cancer cells present elevated GSH levels that generally increase antioxidant capacity and resistance to oxidative stress and regulate different mechanisms linked to carcinogenesis, sensitivity against cytotoxic drugs, ionizing radiation, and some cytokines, DNA synthesis, and cell proliferation (Estrela et al., 2006). There are yet a few reports on the possible role of flavonoids, as well as other phytochemicals, in modulating the glutathione antioxidant system activity, including regulation of GSH intracellular levels through targeting its synthesis (Ramos and Aller, 2008), induction of MRP-1 mediated GSH efflux (Kachadourian and Day, 2006), or inhibition of glutathione peroxidase enzyme activity (Trachootham et al., 2006). Upon grape seed extract treatment, HT29 colon cancer cells showed increased ROS production (that might result in oxidative stress in cells) and a decreased level of intracellular reduced glutathione (Kaur et al., 2011). In addition, after delphinidin and

cyanidin treatment in primary (Caco-2) and metastatic (LoVo and LoVo/ADR) colorectal cancer cell lines, no significant changes in the total GSH levels were observed in Caco-2 and LoVo cells, while both were shown to deplete intracellular glutathione levels in LoVo/ADR cells. GSSG content was not measurable in Caco-2 and LoVo cells, suggesting a normal cellular GSH/GSSG ratio (30:1–300:1) (Cvorovic et al., 2010). Cells undergoing apoptosis appear to rapidly and selectively release GSH into the extracellular space (Ghibelli et al., 1995; Ghibelli et al., 1998; Hammond et al., 2007). Hammond and colleagues demonstrated that apoptotic GSH export is directly linked to MRPs. Indeed basal and apoptotic GSH releases were decreased after RNAi reduction of MRP1 expression in Jurkat cells, indicating that MRP1 is a major player in both processes (Hammond et al., 2007). MRP1-channelled GSH export from cells can be also increased by different xenobiotics, including arsenite, verapamil (VRP), and some naturally-occurring flavonoids (Leslie et al., 2003; Loe et al., 2000).

GSTs are known as a family of Phase II detoxification enzymes that catalyze the conjugation of GSH (S-glutathionylation) with different compounds as xenobiotics and drugs or their metabolites, to form mercapturates (Hayes et al., 2005).

It has been recently shown that anthocyanin fractions from selected cultivars of Georgia-Grown Blueberries at 50-150 íg/mL do induce apoptosis in HT-29 colon cancer cells but these same concentrations decrease GST activities rather than induce it (Srivastava et al., 2007).

There are several studies, in normal cells and tissues, in which it was demonstrated that anthocyanins, probably involving some protein kinases, modulate the activity of some GSH-dependent enzymes, thus ameliorating the antioxidant response (Hou et al., 2010; Suda et al., 2008; Veigas et al., 2008).

GSSG formed intracellularly is continuously reduced to GSH by the activity of GR. If oxidative stress or other factors limit the GR activity (e.g., glucose-6-phosphate dehydrogenase deficiency may limit NADPH supply), GSSG will accumulate (Deneke and Fanburg, 1989). In this respect, Cvorovic and colleagues showed that delphinidin and cyanidin did inhibit GR activity in LoVo/ADR cells but not in Caco2 and Lovo cells (Cvorovic et al., 2010). This has two important consequences: (i) the thiol redox status of the cell will shift, activating oxidant response transcriptional elements; and (ii) GSSG may be preferentially secreted out of the cell. (i) The protein sequences of many transcription factors contain cys residues, mainly localized in the DNA-binding domain that, when oxidized, cause a different modulation of gene expression (Arrigo, 1999). (ii) GSSG may be reduced back to GSH, but when GSSG is present in excess, it is also eliminated from the cell by export into the extracellular space. Strong evidence that this export step is mediated by MRP2 was provided by studies of GSSG transport with canalicular membrane-enriched vesicles derived from normal and EHBR (Eisai hyperbilirubinuric rats) rats (Ballatori et al., 2009).

4.4 Intracellular pH and apoptosis

Despite the genetic variability, two phenotypes common to all tumor cells are cellular alkalinization and a shift to glycolytic metabolism. In the first decade of the 20th century, Otto Warburg found that cancer cells, even in the presence of oxygen disposition and a higher request of ATP for fast growing cells, prefer to metabolize glucose via glycolysis instead of oxidative phosphorylation (Warburg, 1956a). The oxygen levels within a tumor

vary both spatially and temporally. The elevated glycolytic pathway of cancer cells appears to be a response to hypoxia due to the growth of the tumor surpassing the available vascular supplied oxygen (Mathupala et al., 2001) and seems to be controlled directly by the antiapoptotic protein Akt that generates apoptotic resistance *in vitro* (Elstrom et al., 2004).
Then, the decreased dependence on aerobic respiration becomes a selective advantage for survival and proliferation escaping from the apoptotic event. Cell metabolism is shifted toward the increased expression of glycolytic enzymes, glucose transporters, and inhibitors of mitochondrial metabolism that result in a transitional intracellular acidification (Hsu and Sabatini, 2008) and increased glucose uptake is observed coincident with the transition from colon adenomas to invasive cancer (Yasuda et al., 2001). Nevertheless, evidence that intracellular acidification is associated with the progression of apoptosis, has been steadily accumulating (Barry et al., 1993; Gottlieb et al., 1996; Li and Eastman, 1995; Rebollo et al., 1995).
An important role in the intracellular acidification could be due to alterations of membrane pHi-regulating mechanisms, including the Na^+/H^+ exchanger (NHE) that might favor accumulation of the protons produced by energetic metabolism. NHE is ubiquitously expressed transporter in the plasma membrane with a main function to extrude H^+ from the cytoplasm.
Multidrug resistant tumor cells exhibit an altered pH gradient across different cell compartments, which favors a reduced intracellular accumulation of antineoplastic drugs and a decreased therapeutic effect. In fact, the activity and expression of NHE are increased in doxorubicin-resistant (HT29-dx) human colon carcinoma cells in comparison with doxorubicin-sensitive HT29 cells (Miraglia et al., 2005). On the other hand, it was demonstrated that activation of the NHE-1 and the resulting cellular alkalinization play a key role in oncogenic transformation (Reshkin et al., 2000). Cyanidin (10 microM), but not its glycosides, could inhibit the neurotensin- and EGF-induced increased rate of extracellular acidification in HT-29 human colon adenocarcinoma cell line probably by inhibiting cellular metabolism, rather than directly altering Na+/H+ exchange (Briviba et al., 2001).
The effect of anthocyanins on metabolism involved in pH modulation of apoptosis is anyway a poor-trodden path.

5. Roadmap for further investigations

5.1 Role of flavonoids on DNA methylation

In humans, multistage carcinogenesis was previously considered a consequence of genetic alterations that cause activation of oncogenes and inactivation of tumor suppressor genes. In addition to genetic events, epigenetic events are another leading player in carcinogenesis (Link et al., 2010). Indeed, it is believed that majority of cancers result from changes that accumulate throughout the life due to the exposure to various endogenous factors and arguably diet and environment-mediated epigenetic perturbations play a crucial role in cancer progression in humans (Herceg, 2007). It was first recognized more than 25 years ago that in colorectal cancer cells, global DNA methylation patterns differed considerably from those in their normal counterparts (Venkatachalam et al., 2010).
The developmental biologist Conrad H. Waddington coined the term 'epigenetics' in 1942, trying to describe reversible heritable changes in gene expression that occur without alteration in DNA sequence sufficiently powerful to regulate the dynamics of gene expression (Waddington, 1951 as cited in (Hitchler and Domann, 2009).

One of the "epigenome" processes is DNA methylation, a covalent chemical modification resulting in addition of a methyl (CH3) group at the carbon 5 position of the cytosine ring in CpG dinucleotides (Kanai and Hirohashi, 2007). This process plays important roles in chromatin structure modulation, transcriptional regulation and genomic stability, and is essential for the development of mammals (Ducasse and Brown, 2006; Li, 2002). CpG dinucleotides are not uniformly distributed throughout the human genome, but are often enriched in the promoter regions of genes. Short CpG-rich regions are also called as ''CpG islands'', and these are present in more than 50% of human gene promoters and can lead to gene silencing and proliferation or to affect the metabolic processes associated with energy metabolism. (Link et al., 2010). This mechanism is an enzymatic process mediated by DNA methyltransferases (DNMT): DNMT1, also called a "maintenance methyltransferase", preserves existing methylation patterns following DNA replication; DNMT3a and DNMT3b, on the other hand, serve as *de novo* methyltransferases, which act independently of replication on both strands, altering the epigenetic information content (Yu et al., 2011).
Recent studies havedemonstrated that all three DNMTs are overexpressed in several tumor types, including tumors of the colon and rectum, bladder, and kidney. When DNMT1 and DNMT3b are knocked out in colon cancer cell lines, methylation of tumor suppressor genes such as p16 is almost entirely eliminated and the gene is re-expressed (Rhee et al., 2002), as well as it has been established that the inhibition of DNA methyltransferase activity can strongly inhibit the formation of tumors (Stresemann et al., 2006).
It is known that some nutrients like folic acid, B vitamins and SAM (S-adenosylmethionine) and anthocyanins are key components of the methyl-metabolism pathway (Vanzo et al., 2011). Their methyl-donating mechanism can rapidly alter gene expression by modulating the availability of methyl donors as well as DNMT activity (Ross, 2003). There is a growing interest in the role of polyphenols in prevention of DNA methylation. It was demonstrated that epigallocatechin-3-gallate (EGCG), a tea polyphenol, through its methylation exerted by catechol-O-methyltransferase (COMT), indirectly inhibited DNMT. Indeed, S-adenosyl-L-homocysteine (SAH), produced by COMT reaction is a potent inhibitor of DNMT (Fang et al., 2003). On the other hand, EGCG can directly inhibit DNMT through the hydrogen bonds formation with different residues in the catalytic pocket of the enzyme (Lee et al., 2005). Moreover, Fang et al. showed that reactivation of some methylation-silenced genes by EGCG was also demonstrated in human colon cancers and prostate cancer cells (Fang et al., 2003).

5.2 Apoptosis and ATP/ADP ratio

Oxygen consumption in cells is regulated by a respiratory control system which depends on ADP and Pi. When the amount of ATP is high, the amount of ADP is limited and therefore, use of oxygen declines. In other words, oxygen consumption increases as the need for ATP arises (Valle et al., 2010). ATP generation through oxygen conversion is not a fully efficient process because a percentage of the energy of the electrochemical gradient is lost and not coupled to ATP production (Matsuyama and Reed, 2000). This situation arises due to a phenomenon called 'proton leak' which causes protons to return to the mitochondrial matrix via alternative pathways that by-pass ATP synthase (Brand, 1990; Brown and Brand, 1991; Valle et al., 2010). Lynen suggested that the increased dependence of cancer cells on glycolysis stemmed not from their inability to reduce oxygen, but rather from their inability to synthesize ATP in response to the mitochondrial proton gradient (Lynen, 1951 as cited in (Samudio et al., 2009).

Although, some explanations for the 'proton leak' come from the biophysical properties of the inner membrane, much of the explanation comes from the activities of a family of mitochondrial proteins termed uncoupling proteins (UCPs) (Klingenberg, 1999; Valle et al., 2010). UCPs exploit the gap in pH concentration to transfer the proton through the inner membrane into the matrix where they are released. Consequently, the mitochondrial membrane potential decreases, reduction of O_2 via the respiratory chain is no longer linked to ATP synthesis and ATP/ADP exchange is not longer maintained (Vander Heiden et al., 1999).
The influence of anthocyanins on ATP/ADP ratio and on UCPs role could be the aim of further studies.

5.3 Apoptosis and oxygen consumption

Cancer cells seem to show high glycolytic rates even when oxygen is sufficient for oxidative phosphorylation (OXPHOS). This condition leads to a survival benefit of the tumor providing protection from oxidative stress and resulting in apoptosis avoidance (Kondoh et al., 2007a; Kondoh et al., 2007b). The importance of glycolysis in the survival of cancer cells was demonstrated by Bonnet and colleagues. Their experimental approach aimed at inhibiting the anaerobic glycolisys by repressing the activity of pyruvate dehydrogenase kinase (PDK) with dichloroacetate (DCA). PDK acts as a negative modulator of pyruvate dehydrogenase, a gate-keeping mitochondrial enzyme which controls the glucose oxidative fate into the cell. DCA changes the metabolism of cancer cells from the cytoplasm-based glycolysis to the mitochondria- based glucose oxidation. This led to increased ROS production and decreased mitochondrial membrane potential, efflux of pro-apoptotic mediators from mitochondria, and induction of mitochondria-dependent apoptosis only in cancer cells (Bonnet et al., 2007). On the other hand, in the majority of mammalian cells, glycolysis is inhibited by the presence of oxygen, which allows the mitochondria to oxidize pyruvate to CO_2 and H_2O.
The transcription factor p53 regulates cellular energy metabolism and antioxidant defense mechanisms. Emerging evidence has shown that these two functions of p53 contribute greatly to p53's role in tumor suppression (Bensaad and Vousden, 2007; Matoba et al., 2006; Sablina et al., 2005). Loss of p53 results in decreased oxygen consumption and impaired mitochondrial respiration and promotes a switch to high glucose utilization in aerobic glycolysis in cells (Maddocks and Vousden).
It was shown that p53 regulates the OXPHOS dependence of cell by modulating the assembly of a key complex in the mitochondrial electron chain transport: cytochrome c oxidase (COX) (Ma et al., 2007; Matoba et al., 2006). It was demonstrated, in fact, that in HCT116 cells, p53 controls the expression of SCO 2 (Synthesis of Cytochrome c Oxidase 2). SCO2 is required for the assembly of mitochondrial DNA-encoded COX II subunit (MTCO2 gene) into the COX, so, p53 directly regulates mitochondrial oxygen consumption. p53 mutations in cancer cells induce a loss in SCO2, thereby resulting in a switch from an aerobic mitochondrial respiration to anaerobic glycolysis. p53 induces SCO2 expression to enhance mitochondrial respiration and induces TIGAR expression to slow glycolysis (Won et al., 2011).
The metabolic implications of anthocyanins through the oxidative use of glucose could be appreciated indirectly. In fact, it is known that anthocyanins induce p53 expression (Fimognari et al., 2005; Lo et al., 2007; Renis et al., 2008), but a direct involvement of this compounds on glucose metabolic use it is not yet demonstrated.

6. Conclusions

The different observations found in epidemiological studies in comparison to the *in vitro* ones are linked, partly to the relatively low flavonoid intake and complexity of metabolism in humans, and partly to the lack of adequate molecular biomarkers for monitoring the earliest stages of disease development in humans (Pierini et al., 2008). Moreover, the relevance of the *in vitro* studies to the *in vivo* situation needs to be confirmed in view of the high concentrations of polyphenols employed in the *in vitro* studies.

All the data recorded about the role of polyphenols and flavonoids has been obtained through the use of classical cell biology and biochemistry methods. Maybe nutrigenomics, that is the study of the effects of foods and food constituents on gene expression could deepen our understanding of these and other phytochemicals (Corthesy-Theulaz et al., 2005; Davis and Hord, 2005; Mariman, 2006).

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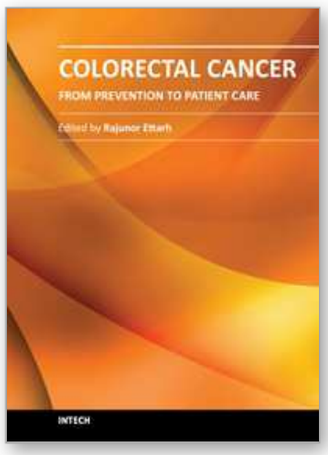
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The projections for future growth in the number of new patients with colorectal cancer in most parts of the world remain unfavorable. When we consider the substantial morbidity and mortality that accompanies the disease, the acute need for improvements and better solutions in patient care becomes evident. This volume, organized in five sections, represents a synopsis of the significant efforts from scientists, clinicians and investigators towards finding improvements in different patient care aspects including nutrition, diagnostic approaches, treatment strategies with the addition of some novel therapeutic approaches, and prevention. For scientists involved in investigations that explore fundamental cellular events in colorectal cancer, this volume provides a framework for translational integration of cell biological and clinical information. Clinicians as well as other healthcare professionals involved in patient management for colorectal cancer will find this volume useful.

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