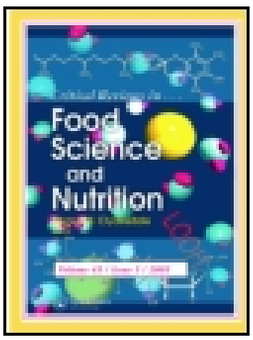

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Dietary polyphenols and chromatin remodelling

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Abstract

Polyphenols are the most abundant phytochemicals in fruits, vegetables and plant-derived beverages. Recent findings suggest that polyphenols display the ability to reverse adverse epigenetic regulation involved in pathological conditions, such as obesity, metabolic disorder,

cardiovascular and neurodegenerative diseases and various forms of cancer. Epigenetics, defined as heritable changes to the transcriptome, independent from those occurring in the genome, includes DNA methylation, histone modifications, and post transcriptional gene regulation by non-coding RNAs. Sinergistically and cooperatively these processes regulate gene expression by changing chromatin organization and DNA accessibility. Such induced epigenetic changes can be inherited during cell division, resulting in permanent maintenance of the acquired phenotype, but they may also occur throughout an individual life-course and may ultimately influence phenotypic outcomes (health and disease risk). In the last decade, a number of studies have shown that nutrients can affect metabolic traits by altering the structure of chromatin and directly regulate both transcription and translational processes. In this context, dietary polyphenol-targeted epigenetics becomes an attractive approach for disease prevention and intervention. Here, we will review how polyphenols, including flavonoids, curcuminoids and stilbenes, modulate the establishment and maintenance of key epigenetic marks, thereby influencing gene expression and, hence, disease risk and health.

Key words:

DNA methylation;

Histone modifications,

MicroRNA;

Epigenome.

1. Introduction

Chronic diseases including diabetes, cancers and cardiovascular disorders are among the leading causes of deaths worldwide and exert a huge impact on national economies. Despite the success of genome-wide studies in identifying loci associated with complex human diseases, a substantial proportion of the causality remains unexplained (Sabatti et al. 2009). Therefore, new studies focused on the discovery of more incisive and cost-effective therapeutic and preventive approaches are needed. Nowadays, there is increasing interest on how nutrients regulate metabolism and how they affect consumers' health. Among the numerous external environmental factors, diet is thought to be one of the most important in consideration of its ability to modify gene expression at transcriptional level (Choi and Friso 2010). Currently, food is considered an environmental condition that shapes the activity of the (epi)genome influencing stress adaptive responses, immune homeostasis, metabolism, and the physiology of the body. Given the responsiveness of epigenetic marks to dietary factors, the new effort is to find new plant compounds that can have an effect on the epigenetic molecular processes in order to use them in disease prevention and treatment.

The term phytochemicals generally indicates a large group (> 10,000) of non-nutritional compounds often associated with the prevention of degenerative pathologies, such as cancer, cardiovascular and neurodegenerative diseases (Kim and Kim 2013, Teiten et al. 2013). The large part of phytochemicals is represented by polyphenols which, in turn, are classified into two major types: flavonoid and non-flavonoid phenolics (essentially phenolic acids, stilbenes and lignans) (Pietta et al. 2003).

Recently, polyphenols captured scientists' attention in consideration of their ability to modulate epigenome. Many studies reported the ability of polyphenols to inhibit DNA methylation and histone modifications or to change the availability of substrates necessary for those enzymatic reactions. By these mechanisms polyphenols may play a significant role in the prevention of different forms of cancer, neurodegenerative, cardiovascular, inflammatory and metabolic diseases (Link et al. 2010, Bilotto et al. 2013, Russo et al. 2014).

The aim of this work is to review the experimental observations showing the role and mechanisms by which dietary polyphenols participate in the control of metabolic processes and modulate epigenetic pathways such as DNA methylation, histone modifications and miRNA expression. We hope, with this review, to highlight the most prominent advance in the field and underline the critical issues, which deserve future investigations.

2. Basic Concepts in Epigenetics

The term "epigenetics" defines processes regulating reversible heritable changes in gene expression that are not accompanied by alterations in DNA sequence and are transmitted through meiosis and mitosis across generations. The epigenetic mechanisms determine whether a gene is silenced or expressed not only in cellular differentiation during embryonic and foetal development but also throughout the whole life course.

The main epigenetic processes are DNA methylation (methylation of cytosine within CpG dinucleotides), post-translational modifications of histones (amongst which acetylation/deacetylation and methylation/demethylation of lysine residues are the most widely studied) and, recently, a variety of non-coding RNAs including microRNAs (miRNAs), which

can bind to and regulate multiple mRNAs (Kim et al. 2009). Together, these processes contribute to gene regulation by changing chromatin architecture and/or access by transcription factors.

When considering how different environmental cues can impact on the genome, it is important to emphasize that epigenetic modifications on DNA and chromatin constitute the link between environment and gene expression. In case of aberrant environmental conditions, the epigenotype may become altered determining modifications in gene expression that results in an altered phenotype. Thus, the phenotype is determined not only by the genotype but also by the epigenotype, which may change during development or in postnatal life due to environmental influences. Evidences support that epigenetic mechanisms may mediate the effects of the diet and other environmental conditions on the exposed individuals and act as determinants of later health outcomes (Zeisel 2009). Indeed, growing evidences suggest that epigenetic plasticity together with genetic modifications are not only involved in cancer, as extensively studied (Vanden Berghe 2012), but also in the development of common complex diseases, such as, diabetes, obesity, neurodegeneration and cardiovascular disease (Petronis 2010).

Epigenetic modifications at selected genes can be reverted: methylation silenced genes can become active by demethylation, and histone complexes can become transcriptionally active through nutrients and dietary interventions that can change the acetylation and methylation status of various histones. Due to its reversibility, epigenetics represent a starting point of clinical intervention, and nutrients can modify the expression of genes playing a critical role in physiologic and pathologic processes through modulation of epigenetic phenomena.

2.1 DNA methylation

DNA methylation involves an enzymatic process by addition of a methyl group to the 5' position of the cytosine ring within CpG dinucleotides to form 5-methylcytosine.

CpG are normally methylated when scattered throughout the genome, but are mostly unmethylated when they are clustered as CpG island at 5' ends of many genes. Silenced and transcriptionally active genes are characterized by hypermethylation or hypomethylation, respectively, of CpG islands within their promoter regions (Suzuki and Bird 2008).

DNA methylation regulates several key physiological processes, including X chromosome inactivation, imprinting and the silencing of germline-specific genes and repetitive elements.

DNA methylation at CpG dinucleotides occurs upon transfer of S-adenosylmethionine (SAM) on cytosine by DNA methyltransferases (DNMTs). The conventional view is that DNMT3A and DNMT3B catalyze the addition of methyl groups *de novo* during embryogenesis while DNMT1 is responsible for maintaining DNA methylation patterns during cell replication.

Although historically thought to be stable, DNA methylation has been recently shown to undergo active demethylation, yielding multiple intermediate forms of CpG modification, including hydroxymethylation, formylation and carboxylation (Ito et al. 2010).

2.2 Post-translational modifications of histones

Within the eukaryotic cell nucleus, genetic information is organized in a highly conserved structural polymer, termed chromatin, which can regulate transcriptional processes through post-synthetic modifications of DNA and the histone (Laskowski and Thornton 2008). The basic unit of chromatin structure is the nucleosome that encompasses 147 bp of DNA wrapped around an octamer of histones, formed by two H3/H4 dimers and two H2A/H2B dimers. The addition of

other proteins, including the linker histone H1, enables the primary conformation to coil into 30 nm fibers or higher-order chromatin structure. Chromatin organization is regulated primarily through dynamic modifications of the histone proteins that can control the packaging of DNA and therefore, strongly contribute to the control of gene expression. The post-translational modifications (PTMs) of histones occur mostly within the amino-terminal histone "tails" that protrude from their surface, but also within the histone globular domain and include acetylation, biotinylation, methylation, phosphorylation, ubiquitination, SUMOylation, ADP ribosylation, proline isomerization, citrulination, butyrylation, propionylation, and glycosylation (Kouzarides 2007).

The majority of these modifications take place at lysine, arginine and serine residues and constitute, together with DNA methylation pattern, the chromatin language or the so-called "histone code" which is read and translated into transcriptional changes (Kouzarides 2007). Such modifications can either be associated with transcriptional activation or transcriptional repression. There is good evidence that di- and trimethylation and poor acetylation of lysine 9 residue on histone H3 are associated with silencing of gene expression. By contrast, acetylation of histones H3 and H4 together with methylation of lysine 4 residue on histone H3 regulate active gene transcription. Post-translational modifications of histones are reversible and dinamically regulated by processes involving various enzymes responsible for adding or removing covalent modifications to histone proteins. As an example, histone acetyltransferases (HATs) and histone deacetylases (HDACs) add and remove, respectively, acetyl groups while histone methyltransferases (HMTs) and histone demethylases (HDMs) add and remove methyl groups, respectively, from histone proteins (Haberland et al. 2009).

2.3 Noncoding RNA

Besides DNA methylation and histone modifications, there is good evidence that also noncoding RNAs (ncRNAs) contribute to the post-transcriptional regulation of genes (Rouhi et al. 2008). MicroRNA (miRNAs), the best-characterized family of small ncRNAs, are single-stranded RNAs, about 19-24 nucleotides in length. They regulates gene expression by sequence specific base pairing with 3'-untranslated regions of the target messenger RNA resulting in degradation or translational inhibition (He and Hannon 2004). miRNAs function by recruiting a protein complex, called RISC (RNA-induced silencing complex), to target gene transcripts that are silenced via degradation of the messenger RNA or by preventing translation. miRNA in metazoans do not need to form a perfect base-pair match to their target site, and thus one miRNA can regulate several genes. miRNAs regulate a large spectrum of biological processes such as cell growth and proliferation, development, differentiation, organogenesis, metabolism, immunity, thus they can influence multiple diseases including obesity, cancer, cardiovascular disease, and diabetes (Iorio and Croce 2012).

3. Dietary polyphenols in the control of epigenetic changes

For many years animal models have been used to demonstrate how environmental exposure to nutrients may change gene expression and alter phenotype through epigenetic modifications. A very good model is the agouti viable yellow (A^{vy}) mouse, which is genetically altered to have yellow fur. Indeed, it has been demonstrated that supplementing the mouse diet with folic acid, a known methyl donor, the level of DNA methylation at the agouti gene increases thereby

changing the color pattern of the hair coat. Interestingly, the A^{vy} mice are also heavier and present an elevate risk for hyperinsulinemia, diabetes and tumors. Supplementing the mothers' diet with methyl donors also prevent the future transmission of obesity to the offspring (Waterland et al. 2008). This clearly shows that a link exists between nutrition, epigenetics, and the resulting phenotype.

Dietary components traditionally are divided into macronutrients and micronutrients. Although several studies implicate that macronutrients play a major role in fetal programming (McMillen et al. 2008), also micronutrients including vitamins and minerals play a critical role. For example, folate, a water-soluble B vitamin can change DNA methylation status by delivering a methyl group for the synthesis of AdoMet, a common co-substrate involved in methyl group transfers.

Polyphenols are nonessential micronutrients described for their capacity to prevent chronic disorders, such as cancer, cardiovascular and metabolic diseases including obesity and type 2 diabetes (Fraga et al. 2010). Their beneficial effect on cardiovascular and metabolic diseases derive from their ability to reduce LDL oxidation, to slow down atherosclerotic lesion development, to decrease blood pressure and to improve insulin resistance (Habauzit and Morand 2012, van Dam et al. 2013). They also regulate apoptosis and prevent angiogenesis and tumor cell proliferation thus exhibiting an anticancer effect (Cimino et al. 2012).

Most of phytochemicals are represented by polyphenols which are divided into several classes according to their chemical structures (Pietta et al. 2003)**.**

- **Phenolic acids** that are largely present in berry fruits kiwi, cherry, apple, pear, chicory and coffee represent a third of the polyphenolic compounds and are divided into two

main classes: hydroxybenzoic acids (C6-C1) (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid) and hydroxycinnamic acids (C6-C3) (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, sinapic acid);

- **Flavonoids** (C6-C3-C6) including six subclasses: antocyanins, flavanols, flavonols, flavones, flavanones, and isoflavones;
- **Lignans** (C6-C3-C3-C6), found in high concentrations in linseed and other grains and cereals, largely known for their phytoesterogenic activity;
- **Stilbenes** (C6-C2-C6), present in the human diet in low quantities with resveratrol as the main studied compounds of these groups and largely detected in grapes and red wine.
- **Curcuminoids** (C6-C3-C1-C3-C6) constitute another class of polyphenols, with curcumin being the main molecule that is found principally in the rhizomes of *Curcuma longa.*

Phytochemicals have been attributed lot of effects in animal cells thanks to their capacity to regulate various enzymes involved in cell metabolism. However, many phytochemicals, including polyphenols, are rapidly metabolized and degraded in the human body (Manach et al. 2009). In fact, it has been already underlined the discrepancy between phytochemical concentrations applied *in vitro* studies (usually tens of micromolars) and those found *in vivo* (human and animal sera), after vegetable and fruit ingestion (usually below 1 μM) (Russo et al. 2010, Spagnuolo et al. 2012).

The epigenome-regulating actions of polyphenols in cancer are recently surfacing (Gilbert and Liu 2010). Some bioactive food components are capable to inhibit the DNA methyltransferase enzymes thus reducing the DNA methylation of key cancer-causing genes. In addition to their

ability to induce changes in DNA methylation, it has been described that dietary polyphenols such as curcumin, resveratrol, and catechism can also influence transcription by altering the post-translational modifications of histones, which, in turn, cause alterations to the chromatin structure. In this context, several compounds are described to possess potent HATs and HDACs inhibitory activities. Moreover, a few reports have suggested that polyphenols may reduce carcinogenesis through miRNA (Saini et al. 2010). Despite experimental evidence shows that these dietary factors may induce epigenetic modifications, the unsolved question regards the molecular mechanisms triggered by these compounds. Since these non-nutrient dietary factors do not generate additional signals and messengers that can indeed modify epigenetic marks their influence on the epigenome will largely depend on their bioavailability.

In the next paragraphs, we will review the recent understanding on the effects of specific dietary polyphenols on epigenetic mechanisms which may have a significant role in future therapeutic applications. We have selected molecules belong to the most representative classes of polyphenols (flavanols, flavonols, isoflavones, curcuminoids and stilbenes) showing a broad range of interference with epigenetic processes.

3.1 Flavonoids

3.1.1 Flavanols

Tea polyphenols have received considerable public attention due to the positive association between tea consumption and beneficial health effects. The putative health benefits attributed to green tea (*Camellia sinensis, Theaceace*) is due to high concentrations of polyphenolic compounds known as catechins, including epigallocatechin-gallate (EGCG), epigallocatechin (EGC), epicatechin-gallate (ECG), and epicatechin (EC).

This group of flavonoids is considered as one of the most promising treatments for metabolic syndrome. EGCG, i.e., has been shown in high fat-fed mice to prevent the development of obesity by stimulating the expression of skeletal muscle genes involved in fat oxidation and by modulating fat absorption from the diet (Sae-tan et al. 2011). In addition, in mice EGCG supplementation decreases liver damages and reduces liver triglyceride, plasma cholesterol and inflammatory cytokines such as MCP-1, CRP or IL-6 (Chen et al. 2011).

The chemopreventive and anticancer effect of green tea polyphenols has been demonstrated by the association between green tea consumption and a lower incidence of gastric, ovarian, breast, colorectal, pancreatic and skin cancers (Li and Tollefsbol 2010). The anti-cancer effects of EGCG are mediated by epigenetic mechanisms. It is well established that EGCG inhibits DNMT by directly binding to the enzyme (Fang et al. 2007). Human esophageal (KYSE 150), colon (HT29), prostate (PC3), and mammary (MCF-7 and MDA-MB-231) cancer cell lines treated with EGCG show hypomethylation and re-expression of several tumor suppressor genes including $p16^{INK4\alpha}$, *RARβ, MGMT* and the DNA mismatch repair gene (*hMLH1*) as a consequence of a decrease in DNMT1 activity (Fang et al. 2007). By contrast, other authors reported the lack of demethylation and activation of several genes by EGCG. As an example, consumption of a green tea extract (0.3%) in drinking water did not induce DNA hypomethylation in murine prostate tissue as demonstrated by a genome-wide DNA methylation profiling assay (Morey Kinney et al. 2009). In this case, the lack of DNMT inhibition by the green tea extract could be probably due to low bioavailability and stability of polyphenols leading to insufficient retention in prostate tissue.

EGCG not only modifies DNA methylation, but produces also its effect as histone modifiers. Indeed, the chemopreventive effect of EGCG has been associated to its capacity to interfere with the enzymatic activities of HDAC, HAT and Class III HDAC sirtuins (SIRT), which modulate inflammation in cancer. Choi et al suggested that EGCG acts as an inhibitor of HAT enzymes, particularly of the p300/CREB-binding protein (CBP) that is associated with cancer growth and survival (Choi et al. 2009).

In the last decades, HDAC inhibitors (HDACi) have been proposed as a new class of drugs capable to induce tumor cell arrest, differentiation, and apoptosis. Many authors started to investigate EGCG as a potential HDAC inhibitor. Pandey and co-workers found that green tea polyphenols increase H3 and H4 acetylation through the inhibition of HDAC activity and reduction of HDAC1, 2 and 3 expression levels (Pandey et al. 2010). By contrast, other authors were not able to demonstrate an activity on these enzymes (Nair et al. 2008, Choi et al. 2009). The epigenetic effect of EGCG has also been recently demonstrated in RKO, HCT-116 and HT-29 colorectal cancer cells and breast cancer cells where EGCG associated to sodium butyrate, a dietary microbial fermentation product of fiber, is capable to induce apoptosis and cell cycle arrest by decreasing HDAC1, DNMT1, survivin and HDAC activity (Saldanha et al. 2014).

Recently, EGCG has been found to have an effect on miRNA in human cancer cells (Tsang and Kwok 2010). In both human malignant neuroblastoma SK-N-BE2 and IMR-32 cell lines 50 μM EGCG have been demonstrated to decrease the expression of oncogenic miRNAs (miR-92, miR-93, and miR-106b) and to increase the expression of tumor-suppressor miRNAs (miR-7-1, miR-34a, and miR-99a) (Chakrabarti et al. 2012). In the mouse lung adenocarcinoma cell line CL13, 40 μM EGCG decrease cell growth by stimulation of miR-210 expression, suggesting that the

anticancer activity of this flavonoid can be determined by the modulation of miRNAs concentrations (Wang et al. 2011).

3.1.2 Flavonols

Quercetin is the major flavonoid present in different foodstuffs, such as onions, apple, or wine and exhibits a wide range of biological effects.

The anticancer effects of quercetin have been demonstrated in animal models (Russo et al. 2012). However, the precise mechanisms of quercetin action are still unclear. At a concentration of 100 μM quercetin decreases the DNA methylation levels of the estrogen receptor (Er-β), $p16^{INH4\alpha}$ and RASSF1A in bladder cancer cells inhibiting cell growth (Ma et al. 2006). It must be noted the extremely high concentration of quercetin used in this study. Tan and collaborators also demonstrated that quercetin is able to inhibit the growth of RKO cells, a human colon cancer cell line via demethylation of the $p16^{INH4\alpha}$ gene promoter which results in enhanced gene expression (Tan et al. 2009). All these data suggest that the antitumor property of quercetin derives from its ability to restore the expression of tumor suppressor gene by changing the DNA methylation levels. Moreover, quercetin can regulate gene expression through changes in histone modifications, i.e. quercetin is able to activate the NAD^+ dependent histone deacetylase SIRT1 via the phosphorylation of AMP-activated protein kinase (Chung et al. 2010).

Quercetin possesses a HAT-inhibitory effect. Indeed, in human endothelial cell it is capable to suppress COX-2 mRNA and protein expression by inhibiting p300 histone acetyltransferase activity (Xiao et al. 2011). In addition to the HAT-inhibitory effect of quercetin, a recent study also suggest a possible HDAC-inhibitory effect (Lee et al. 2011). In this work, the authors

demonstrate that in human leukemia HL-60 cells, quercetin induces FasL-related apoptosis through promotion of histone H3 acetylation and inhibition of HDACs.

The anti-inflammatory effect of quercetin has been also recently linked to changes in miRNA expression. For example, quercetin, supplemented in the high-fat diet of C57BL/6J mice increases expression of miR-125b, a negative regulator of inflammatory gene expression, and miR-122, involved in lipid metabolism and pathogenesis of liver diseases (Boesch-Saadatmandi et al. 2012). Human studies also demonstrate that the expression of 56 miRNAs (and in particular miR-146, miR-125, miR-26, and miR-17) is significantly different in lung cancer from individuals who consumed high versus low quercetin-rich foods. These data suggest that miRNA represent potential targets of quercetin underlying their health effects (Lam et al. 2012).

3.1.3 Flavanones

Flavanones are polyphenolic compounds highly and almost exclusively present in citrus. Clinical and experimental observations have shown that these substances, including hesperidin and naringin, are particularly active in cardiovascular disease prevention (Chanet et al. 2012). Although their antihypertensive, lipid-lowering, insulin-sensitizing, anti-oxidative, and anti-inflammatory properties have been recently recognized, the mechanisms responsible for flavanone actions have not been fully elucidated.

Recently, it has been demonstrated that the combined treatment of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) with naringenin, synergistically induces cytotoxicity in neuroblastoma, but not in non-malignant cells (Ling et al. 2012). These data suggest the potential addition of naringenin to SAHA for future clinical trials in cancer patients.

One study reported that hesperidin and naringein supplementation in apolipoprotein E-deficient mice affects the expression of 97 and 69 miRNAs respectively, with 31 miRNAs in common. These data suggest that miRNA could be also potential targets of flavanones underlying their health effects (Milenkovic et al. 2012).

3.1.4 Isoflavones

Soybean isoflavones have been identified as phytochemical therapeutic flavonoids for the treatment of colon-rectal cancer and are considered epigenetic modulator of DNA methylation and histone modifications (Li and Chen 2011).

Genistein is one of the many phytoestrogens contained in soybeans and it is becoming clear that its chemopreventive effect on cancer cell growth derives from its ability to inhibit the DNMT activity, and for this reason, it can be used as antineoplastic drug in some malignancies (Fenaux et al. 2009). In MDA-MB-468 breast cancer cells, genistein partially demethylates the promoter of the GSTP1 tumor suppressor gene (King-Batoon et al. 2008). In renal and prostate cancer, genistein treatment reactivates the tumor suppressor gene B-cell translocation gene 3 (*BTG3*), by inducing promoter demethylation and histone modifications on its promoter (Majid et al. 2009, Majid et al. 2010).

In mice, maternal genistein supplementation during early embryonic development increases methylation in a retrotransposon upstream of the transcription start site of the Agouti gene and determines a shift of the color coat in heterozygous yellow agouti (Avy/a) offspring (Dolinoy et al. 2006). In this model, genistein, by altering the epigenome, decreased ectopic Agouti expression and protected offspring from obesity in adulthood. Similarly, in nonhuman primates,

soy protein and isoflavones, modifying the DNA methylation patterns in liver and muscle, improve body weight, insulin sensitivity and lipid profiles (Howard et al. 2011).

The effects of genistein and other isoflavones on DNA methylation have also been tested in humans. Healthy pre-menopausal women that received either 40 mg or 140 mg isoflavones daily showed increased methylation of two tumor suppressor genes, RARβ2 and CCND2, known to be methylated in breast cancer (Qin et al. 2009). The increased methylation of tumor suppressor genes may indicate possible adverse epigenetic changes, suggesting that isoflavones administration may be contraindicated for women at high risk of breast cancer or breast cancer patients due to its estrogen-like effects.

Genistein possesses the highest histone modifying activity when compared to other isoflavones, such as biochin A and diadzein. For example, in precancerous and cancer breast cells genistein treatment significant increases enrichment of the chromatin activators acetyl-H3 and trimethyl-H3K4, and decreases the chromatin repressor marks, trimethyl-H3K9 and trimethyl-H3K27, in the promoters of $p21^{WAF1}$ and $p16^{INK4\alpha}$ (Li et al. 2013). The increased histone acetylation observed may be the consequence of an increased histone acetyltransferase activity (Majid et al. 2008, Majid et al. 2009, Majid et al. 2010) or of a decreased activity of HDAC (Majid et al. 2009, Majid et al. 2010). In addition, it has been reported that genistein inhibits the expression of SIRT1 (Kikuno et al. 2008).

In addition, it has been suggested that genistein inhibits proliferation, invasion and metastasis of cancer cells via the deregulation of miRNAs (Li et al. 2009). Several studies reported an effect of genistein via modulation of miRNA expression levels in ovarian cancer cell lines (Parker et al.

2009), in pancreatic cancers (Li et al. 2009) and in human uveal melanoma cell lines (Sun et al. 2009).

In conclusion, these studies indicate a potential role of isoflavones in cancer treatment, which could in part be due to deregulation in the expression of specific miRNAs and their respective targets.

3.2 Curcuminoids

Curcumin

Curcumin (diferuloylmethane, a principal and active component of turmeric) is a yellow-colored polyphenolic pigment obtained from the rhizomes of the plant, *Curcuma longa*. Curcumin has strong anti-inflammatory, antibacterial, antiviral, antifungal, antitumor, antispasmodic and hepato-protective effects (Aggarwal et al. 2003).

Liu and co-workers have demonstrated that curcumin and one of its major metabolites, tetrahydrocurcumin can inhibit the enzymatic activity of the DNMT Sss1, an analog of DNMT1, blocking a catalytic group in DNMT1 (Liu et al. 2009). Genomic DNA extracted from a leukemia cell line exposed to curcumin present a decrease in global DNA methylation compared with untreated basal methylation levels (Liu et al. 2009). These data indicate that curcumin is a potent DNA hypomethylating agent, consistently with its broad activity against inflammation, cancer and other disease.

In addition, several studies performed on *in vitro* cancer cell models showed that curcumin could be considered as a strong modulator of HDAC or HAT activity (Teiten et al. 2013). Curcumin

has been identified as a specific inhibitor of p300/CBP activity, and the down-regulation and inhibition of p300 can be responsible for the clinical benefits of curcumin in cancer treatment (Wang et al. 2008). Moreover, in medulloblastoma cell lines it has been demonstrated that curcumin possesses a HDAC-inhibitory activity: in this model, curcumin treatment is associated with down-regulation of HDAC4 protein expression (Chen et al. 2007, Lee et al. 2011).

Given to its ability to suppress 3T3-L1 differentiation and to induce apoptosis, curcumin been considered for a potential application in obesity treatment (Ejaz et al. 2009). In fact, supplementation with curcumin to HFD (high fat diet)-fed in mice results in reduced body weight gain and adiposity, probably as consequence of an increased oxidation and decreased fatty acid esterification in adipose tissue (Ejaz et al. 2009). The epigenetic mechanisms underlying these effects are still unknown given to very few studies analyzing the involvement of curcumin and epigenetic changes in obesity models.

An interesting study by Yun and collaborators highlights the potential role of curcumin in reducing hyperglycemia-induced cytokine production in monocytes via epigenetic mechanisms involving the expression of the HAT enzyme CBP/p300 and the activation of HDAC2 (Yun et al. 2011).

Curcumin also regulates miRNAs to control the expression of their target genes. Microarray analyses of miRNAs expression in pancreatic cancer cells exposed to curcumin led to the up-regulation of 11 miRNAs and the down-regulation of 18 miRNAs (Sun et al. 2008). The potential role of curcumin on cancer cells mediated by epigenetic modulation of miRNAs, is however limited because of its poor bioavailability.

3.3 Stilbenes

Resveratrol

Resveratrol (3, 4'-5-trihydroxy-*trans*-stilbene) has been identified in over 70 plant species. A number of studies have shown the benefits of resveratrol for a variety of degenerative diseases including heart disease, neurological and metabolic disorders. (Smoliga et al. 2011, Vang et al. 2011). It has also been associated to the positive outcomes of caloric restriction (CR) (Camins et al. 2010).

Resveratrol represents a good example on how the same natural compound, acting on different epigenetic mechanisms, can play a role in pathologies of different type and origin. In a salt-sensitive model of Wistar rats, hypertension was induced by using the deoxycorticosterone acetate (DOCA). Here, resveratrol treatment (50 mg/l in drinking water) prevents DOCA-induced hypertension, causing an alteration in the epigenetic modification of vessels, namely histone 3 lysine 27 methylation in the aorta and renal artery sections (Han et al. 2015). Resveratrol and pterostilbene prevent the increase in adipose tissue weight following obesogenic diet induced in rats, inhibiting up-regulation of fatty acid synthase (FAS) by acting on the methylation status (position -90 and -62 bp) of FAS gene (Gracia et al. 2014).

In sporadic Alzheimer's disease (AD) process, resveratrol may indirectly inhibit pro-inflammatory NF-κB-regulated miRNA. In fact, blocking NF-κB with resveratrol may have some therapeutic value in reducing inflammatory neurodegeneration (Lukiw 2012).

The effects of resveratrol on cancer prevention and treatment via the interference on epigenetic changes have been largely documented (Link et al. 2010, Stefanska et al. 2012). In lung cancer,

resveratrol down-regulates the forkhead box C2 (*FOXC2*) gene, which is critical for suppression of tumor metastasis in *in vitro* and *in vivo* models. These effects occur via the inhibition of miRNA-520h, which blocks the signal cascade miRNA-520h⊣PP2A/C⊣Akt → NF-κB → FOXC2 resulting in decreased expression of FOXC2 (Ying et al. 2013). Resveratrol has been recently described as an activator of the silent information regulator T1 (SIRT1) gene that increases AMP-activated protein kinase (AMPK) phosphorylation. In prostate cancer cells, activation of SIRT1 by resveratrol led to an abrupt decrease in H2A.Z, a protein which in its acetylated form is implicated in oncogene up-regulation (Baptista et al. 2013). Probably, resveratrol mediates its activating effects on SIRT1 by increasing its interaction with laminin A (a major nuclear matrix protein), thus aiding in the nuclear matrix localization of SIRT1 (Ghosh et al. 2013). In a rat model of 17β-estradiol (E2)-induced breast carcinogenesis, resveratrol exerts protective effects via up-regulation of the nuclear factor erythroid 2 (*NRF2*) through the inhibition of its promoter methylation and block of the increase of miR-93 induced by E2 (Singh et al. 2014).

4. Research priorities and conclusions

The aforementioned studies convincingly show that dietary polyphenols may indirectly modulate the epigenome by interfering with specific epigenetic processes leading to changes in chromatin modification patterns and/or correcting aberrant expression of miRNA. However, the molecular mechanisms triggered by polyphenols to induce epigenetic changes are largely unknown and represent a priority for further investigation. In addition, most of these studies address candidate

genes, so that relatively little is known about which epigenomic marks are most labile in response to exposure to dietary polyphenols (McKay and Mathers 2011).

A paradox of many *in vitro* studies, included few of the above cited, is represented by the high concentrations of the aglycones applied (up to 100 μM) to levels that will never reached after oral supplementation or after intravenous injection, in case of therapeutic applications. These limits are the result of two common features sheared by many polyphenols: scarce bioavailability and metabolic transformations with the generation of a large number of conjugated derivatives. In fact, after absorption, polyphenols are firstly metabolized in the cells of the small intestine; here, the molecules are conjugated generally to methyl or sulfate groups and/or glucuronic acid to generate metabolites, which are found in the bloodstream. As a result, free aglycones, i.e. the chemical forms normally added to cell lines in *in vitro* study, are not detectable *in vivo*.

If this is the case, how is it possible to explain the encouraging results described above and reported in several reviews on the epigenetic diet based on polyphenols supplementation, especially in cancer prevention? Two main hypotheses can be formulated, both supported by experimental data accumulated in studies on specific compounds: (i). their conjugated forms are biologically active; (ii). after cellular uptake, metabolites are de-conjugated intracellularly regenerating the free aglycone. As an example, de-conjugation of quercetin can occur in liver, in fibroblasts, in human neutrophils during inflammation leading to reactivation of the molecule (Russo et al. 2014).

However, these considerations do not solve the key questions mentioned above: how polyphenols, or their metabolites, modulate, at molecular level, the epigenetic changes. How is it possible that compounds structurally different can exert comparable biological activities acting

on the same cellular targets (i.e., HDACs, HATs or HMTs)? An attractive, although still speculative, explanation can be formulated considering the well-known antioxidant capacity of polyphenols. This class of compound, especially flavonoids, are potent antioxidants since their capacity to stabilize radicals formed after scavenging several ROS (Reactive Oxygen Species) molecules owing to extensive electron delocalization on multiple mesomeric structures existing for the aroxyl radical species of flavonoids (Russo et al. 2012). However, the bifrons chemical nature of polyphenols led to the demonstration that these compounds possessed both antioxidant and pro-oxidant actions. As an example, quercetin accelerates the generation of hydroxyl radicals (OH·) from H_2O_2 with an 8-fold increase in OH· formation at 100 μM in rat liver microsomes (Laughton et al. 1989). Following a recent review on the function of nutritional antioxidants, doubts have been expressed by the authors on the possibility that free radicals can be efficiently scavenged under biological conditions by naturally occurring compounds (Forman et al. 2014). In fact, thousands of phytochemicals, assayed as pure molecules or in extracts, have been shown to have significant free radical scavenging antioxidant capacity, when radicals are produced *in vitro*. These analytical systems may have misled what really happens *in vivo* where intracellular concentrations of candidate antioxidants are extremely low (Forman et al. 2014). The antioxidant effects of phytochemicals may derive from their electrophilic nature; in fact, many of them can be oxidized to electrophilic hydroquinones and quinones during their reaction with free radicals and, in this form, can react with and oxidize specific cysteine residues in Keap1, the factor which binds and docks the transcription factor Nrf2 into the cytoplasm in its inactive form (Turpaev 2013). Upon the alkylation of critical cysteine residues on Keap1, Nrf2 is liberated, translocates to the nucleus and binds the electrophile response element (EpRE) which, in turn, drives the

transcription of antioxidant defense genes. Therefore regulation of Nrf2-ARE (Antioxidant Response Element) pathway may represent a common mechanism undertaken by dietary polyphenols to maintain the protective nature of Nrf2-ARE pathway which is often hijacked in a number of pathological conditions by means of epigenetic alteration and other mechanisms (Ma and He 2012). Example in this direction are starting to appear in the literature. A recent study suggests that the Keap1-Nrf2-EpRE pathway is both affected by and mechanistically involved in epigenetic activities of isothiocyanates (Gerhauser 2013). This hypothesis also conciliates the dichotomy between biological effects of polyphenols and their limited bioavailability mentioned above. In fact, the concentration of the polyphenols can never be sufficient *in vivo* to remove a significant portion of the free radicals, but they are oxidized to electrophilic hydroquinones and quinones during their reaction with free radicals without reaching a toxic concentrations. In other words, the concentrations of electrophiles obtained *in vivo* are sufficient to cause the activation of signaling pathways in cells. In this respect, the limited bioavailability of most polyphenols turns to be a positive characteristic, rather than a limitation (Forman et al. 2014).

Despite these considerations, still key issues must be clarified in future studies: (i) the identification of the active metabolite of the phytochemical that is responsible for its pharmacological actions; (ii) the potential reversibility of the phytochemicals with minimal side effects; (iii) precise cellular targets (receptors or proteins) for either the parent phytochemical molecule or its cellular metabolites under normal physiological and pathological states. Indeed, there is some concerns that epigenetic therapy with dietary inhibitors in long term treatment setups may suffer from lack of specificity (Mai et al. 2008). However, additional preclinical and clinical data are required to better define the effect of the epigenetic changes induced by some of

the dietary polyphenols. Therefore, the next and more important step should be to establish the effective doses and the safety profile of timing of exposure of dietary polyphenols in order to have a beneficial effects in human subjects. For example, timing seems to be crucial in determining the impact of genistein, the primary isoflavone in soy, on metabolic homeostasis in adulthood. In a recent study it has been demonstrated that genistein exposure during the early postnatal period favors the development of obesity in female, but not male rats (Strkovsky et al. 2014). These data suggest that developmental genistein exposure in rats has gender-specific effects on adiposity and underscores the importance of considering timing of exposure and gender when establishing safety recommendations for early-life dietary polyphenols intake. It is now relevant that the medical benefits of dietary polyphenols as epigenetic modulators, especially with respect to their chronic use as nutraceutical agents, will rely on our further understanding of their epigenetic effects during embryogenesis, early life, aging, as well as through different generations.

Considering the plasticity of epigenetic marks and how they respond to dietary factors, there is a need and space for more systematic research and intervention studies aimed to identify those dietary factors which have the most influence in epigenetic marks and to develop an understanding of the mechanisms through which this occurs.

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Table. Summary of polyphenols effects on epigenetic mechanism.

Nutritional factors	**Activity on DNA methylation** *In vitro* **and** *in vivo* **models**	**Activity on histone modifications** *In vitro* **and** *in vivo* **models**	**Activity on miRNA regulation** *In vitro* **and** *in vivo* **models**	**References**
Flavanols (Epigallocatechin-gallate [EGCG])	DNMT inhibitor (human esophageal, KYSE 150; colon, HT29; prostate, PC3; mammary, MCF-7 and MDA-MB-231; cancer cell lines; mice prostate tissue)	HAT, HDAC, SIRT inhibitors (RKO, HCT-116 and HT-29 colorectal cancer cells, breast cancer cells.)	Decreased oncogenic miR-92, miR-93 and miR-106b, and increased tumor-suppressor miR-7-1, miR-34a, and miR-99a (human neuroblastoma SK-N-BE2 and IMR-32 cell lines). Increased miR-210 (mouse lung adenocarcinoma cell line CL13).	(Fang et al. 2007, Wang et al. 2008, Choi et al. 2009, Morey Kinney et al. 2009, Pandey et al. 2010, Chakrabarti et al. 2012, Saldanha et al. 2014)

Flavonols (Quercetin)	DNMT inhibitor (bladder cancer cells; colon cancer cells, RKO).	SIRT1 activator, HAT and HDAC inhibitor (human endothelial cells; human leukemia HL-60 cells).	Increased miR-125b and miR-122 expression (C57BL/6J mice). Microarray analysis: regulation of miR-146, miR-125, miR-26 (human lung cancers).	(Ma et al. 2006, Tan et al. 2009, Chung et al. 2010, Lee et al. 2011, Xiao et al. 2011, Boesch-Saadatmandi et al. 2012, Lam et al. 2012)
Flavanones (Hesperidin and naringin)	DNMT inhibitor (evidence less robust)	Naringenin enhanced the anticancer effect	Microarray analysis: 31 miRNAs regulated by both	(Fang et al. 2007, Ling et al. 2012,

		of the histine deacetylase inhibitor SAHA (neuroblastoma cells)	hesperidin and naringenin. (Apolipoprotein E-deficient mice)	Milenkovic et al. 2012)
Isoflavons (Genistein)	DNMT inhibitor (breast cancer cells). Increases DNA methylation (mice, nonhuman primates, humans).	HAT activator, HDAC and SIRT1 inhibitors Precancerous breast cells and breast cancer cells.	Modulation of miRNAs expression levels (ovarian cancer cell lines, pancreatic cancer cells, human uveal melanoma cell lines).	(Dolinoy et al. 2006, Kikuno et al. 2008, King-Batoon et al. 2008, Majid et al. 2008, Li et al. 2009, Majid et al. 2009, Parker et al. 2009, Qin et al. 2009, Sun et al. 2009, Majid et al. 2010,

				Howard et al. 2011, Li et al. 2013)
Curcuminoids (Curcumin)	DNMT1 inhibitor (leukemia cell line)	HAT and HDAC inhibitor (medulloblastoma cell lines). HAT and HDAC2 activator (THP-1 monocytes).	Microarray analysis: 11 miRNAs upregulated and 18miRNAs down regulated (pancreatic cancer cells).	(Chen et al. 2007, Sun et al. 2008, Wang et al. 2008, Liu et al. 2009, Lee et al. 2011, Yun et al. 2011, Teiten et al. 2013)
Stilbenes (Resveratrol)	Acting on the methylation status of FAS gene (rat adipose tissue).	SIRT1 activator (prostate cancer cells)	Inhibition of miRNA-520h (lung cancer)	(Baptista et al. 2013, Ghosh et al. 2013, Ying et al. 2013, Gracia et al. 2014, Singh et al. 2014)

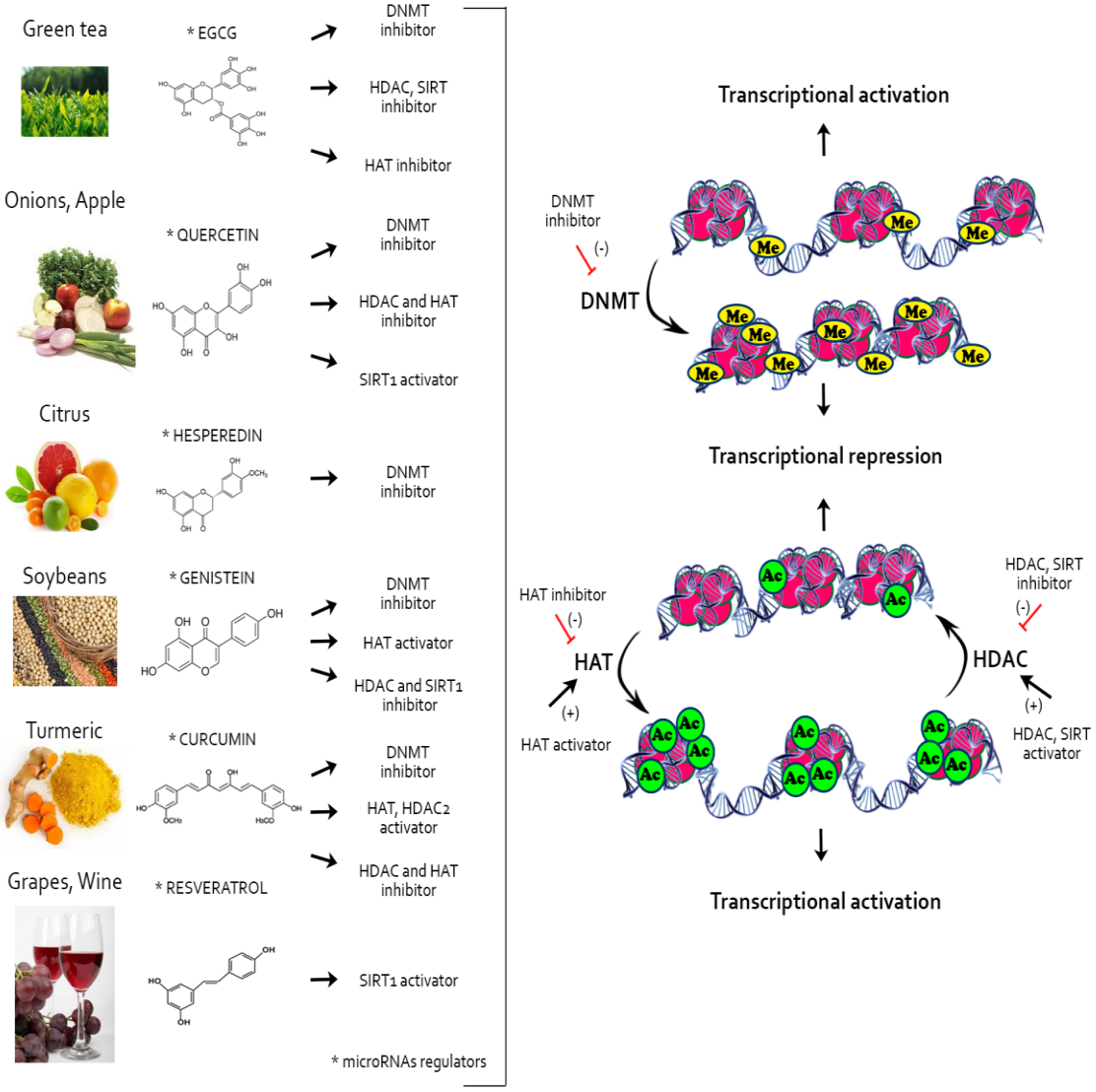

Fig.1. Epigenetic modifications mediated by dietary polyphenols.

The cartoon reproduces chemical structure and main dietary sources of polyphenols (left panel) able to modulate chromatin modifier enzymes (right panel), thereby producing changes in chromatin organization, gene activation, silencing and other nuclear functions. HAT, Histone

Acetyltransferase; HDAC, Histone Deacetylase; DNMT, DNA Methyltransferase; Ac, histone acetylation; Me, DNA methylation.

See text for description.

