Present and future of anti-ageing epigenetic diets

Maria Giulia Bacalini\textsuperscript{a,b}, Simonetta Friso\textsuperscript{c}, Fabiola Olivieri\textsuperscript{d,e}, Chiara Pirazzini\textsuperscript{a,b}, Cristina Giuliani\textsuperscript{1}, Miriam Capri\textsuperscript{a,b}, Aurelia Santoro\textsuperscript{a,b}, Claudio Franceschi\textsuperscript{a,b}, Paolo Garagnani\textsuperscript{a,b,n}

\textsuperscript{a} Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Via San Giacomo 12, 40126 Bologna, Italy
\textsuperscript{b} Interdepartmental Center “L. Galvani” (CIG), University of Bologna, Piazza di Porta San Donato 1, 40126 Bologna, Italy
\textsuperscript{c} Department of Medicine, University of Verona School of Medicine, Policlinico "C.B. Rossi," P.le LA. Scuro 10, 37134 Verona, Italy
\textsuperscript{d} Department of Clinical and Molecular Sciences (DISCLIMO), Universit\`{a} Politecnica delle Marche, Via Tronto 10A, 60020 Ancona, Italy
\textsuperscript{e} Center of Clinical Pathology and Innovative Therapy, I.N.R.C.A. National Institute, Via Birarelli n. 8, Ancona, Italy
\textsuperscript{1} Department of Biological, Geological and Environmental Sciences (BIGEA), Laboratory of Molecular Anthropology & Centre for Genome Biology, University of Bologna, Via Selmi 3, 40126 Bologna, Italy

\textbf{ARTICLE INFO}

Article history:
Received 24 June 2013
Received in revised form 6 December 2013
Accepted 20 December 2013
Available online xxx

Keywords:
DNA methylation
miRs
Ageing
Nutrition
Inflammaging

\textbf{ABSTRACT}

The rapid technological advancements achieved in the last years have boosted the progressive identification of age-associated epigenetic changes. These studies not only contribute to shed light on the molecular basis of ageing and age-related diseases but, given the plasticity of epigenetic modifications, also provide the basis for anti-ageing interventions to counteract the onset of age-related diseases. In this review we will discuss nutritional interventions as a promising approach that can positively counteract epigenetic changes associated with ageing and promote the health for the elderly. First, we will give an overview of age-associated epigenetic signatures, focusing on DNA methylation. Then, we will report recent evidences regarding the epigenetic changes induced by nutritional interventions in the adulthood (referred as “epigenetic diets”), such as (i) caloric/dietary restriction, (ii) diet supplementation with nutrients involved in one-carbon metabolism and (iii) diet supplementation with bioactive food components. Attention will be drawn on the limits of current studies and the need of proper human models, such as those provided by the ongoing European project NU-AGE. Finally, we will discuss the potential impact of epigenetic diets on inflamming and age-related diseases, focusing on cardiovascular disease, highlighting the involvement of epigenetic modifications other than DNA methylation, such as microRNA.

© 2014 Published by Elsevier Ireland Ltd.

1. Introduction

Epigenetic modifications are, by definition, heritable but reversible changes in chromatin structure and gene function. This means that, although epigenetic signatures are transmitted across cellular divisions and – in some cases – also transgenerationally, they can be modified by a wide range of environmental cues, whose effect is in turn dependent on the genetic background of the organism. Epigenetic changes are pivotal in the establishment of developmental patterns in the early life stages, but they appear also to be both triggers and consequences of the phenotypic alterations that characterize ageing. In the past decades a great effort has been placed in order to identify the epigenetic landscapes of ageing and age-related diseases and to interpret them in the framework of ageing theories. From these studies a complex scenario emerges, in which a general relaxation in the mechanisms controlling epigenetic modifications coexists with systemic changes at specific loci, suggesting that an epigenetic program originating during development contributes at least in part to the ageing process (de Magalhães, 2012). Given their tissue-specificity, epigenetic modifications could be also be able to provide consistent hints regarding the “mosaic of ageing” theory (Cevenini et al., 2008), which states that different tissues/organs age at different rates and differently contribute to the ageing of the whole organism. In addition, the identification of age-related epigenetic

\textbf{Abbreviations:} aDMR, age-associated differentially methylation regions; DNMTs, DNA methyltransferases; ROS, reactive oxygen species; CR, caloric restriction; DR, dietary restriction; ADF, alternate-day fasting; PBMCs, peripheral blood mononuclear cells; AdoMet, S-adenosylmethionine; LINE-1, long interspersed element-1; Treg, regulatory T cells; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; miRs, microRNAs; DCs, dendritic cells; Hcy, homocysteine; AdoHcy, S-adenosylhomocysteine; CAD, coronary artery disease.

\textsuperscript{1} Corresponding author at: Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Via San Giacomo 12, 40126 Bologna, Italy. Tel.: +39 0512094748; fax: +39 0512094748.
\textit{E-mail address: paolo.garagnani2@unibo.it} (P. Garagnani).

http://dx.doi.org/10.1016/j.mad.2013.12.006
changes has two important practical consequences. On one side, epigenetic signatures have emerged as potential biomarkers of biological age. On the other side, the malleability of epigenetic modifications has prompted the search for interventions that, acting on epigenetic regulators, could delay or even revert the aged phenotype (Johnson et al., 2012; Rando and Chang, 2012; Vaiserman and Pasyukova, 2012).

A growing number of studies suggest nutrition as one of the most promising anti-ageing interventions (Choi and Friso, 2010; Ford et al., 2011; Ribarić, 2012). Indeed, specific diet components have proved to directly regulate the activity of enzymes that catalyze epigenetic modifications and diet habits can alter the establishment and the maintenance of epigenetic patterns by modifying the intracellular metabolic state or the cellular microenvironment itself.

In this paper we will review the epigenetic changes that occur with ageing and we will provide examples of how nutritional interventions in adulthood, such as that scheduled in the European project NU-AGE (see Box 1), could revert these changes and positively impact the ageing process. Among the broad number of epigenetic modifications, we will specifically focus on DNA methylation, as its role in ageing has been extensively investigated and its responsiveness to nutritional stimuli is the object of several studies. The impact of diet on other age-associated epigenetic modifications, including histone modification, has been deeply reviewed elsewhere (Choi and Friso, 2010; Cosentino and Mostoslavsky, 2013; Huidobro et al., 2013; Li et al., 2011a,b; Martin et al., 2013). Here we will discuss the role of DNA methylation changes during ageing and how DNA methylation patterns can be modified by caloric restriction and by diets enriched in bioactive food components. In this framework we will report current knowledge regarding the contribution of DNA methylation and other epigenetic factors, such as microRNA, to inflammaging, a low-grade systemic inflammation that characterizes the aged phenotype, and their modulation by epigenetic diets. Finally we will discuss how age-related diseases can benefit from nutritional interventions that target epigenetic mechanisms, using cardiovascular disease as example.

Box 1. The challenge of the European project NU-AGE.

As it has been reported in the previous paragraphs, there is a growing amount of data indicating that a series of nutrients, bioactive dietary components and changes in diet habits could modulate the epigenetic pattern impinging upon a series of organs, systems and vital functions that as a whole determine the health status of people at all the stages of life and particularly in elderly. Nevertheless, there is a lack of knowledge regarding the effects of the whole diet on the health status, as the great majority of the past studies have dealt with the effects of a single or few nutrients. Now remarkable insights regarding that relationship between nutrition, epigenetic modifications and ageing can be provided by the European project NU-AGE (FP7, n° 266486; www.nu-age.eu). The rationale and the design of the project are deeply described in the paper by Santoro et al. (in this special issue). Briefly, the NU-AGE project will study in a comprehensive and integrated way the effect of a modified/fortified Mediterranean diet (described by Berendsen et al., in this special issue), specifically designed according to the nutritional needs of people over 65 years of age, on a series of cellular and molecular pathways including epigenetics.

NU-AGE will offer an extraordinary opportunity to elucidate the modulatory role of nutrition on DNA methylation patterns during ageing, considering also the genetic variability (polymorphisms) of some pivotal genes involved in the diet/meta- bolic response.

2. DNA methylation patterns in human ageing

2.1. DNA methylation

DNA methylation is a covalent modification consisting in the addition of a methyl group to the carbon 5 of a cytosine residue, which usually lies at the 5′ of a guanine residue (CpG dinucleotide). The distribution of CpG dinucleotides in the genome is not random, as they are relatively depleted from the bulk of the genome and enriched at the promoters of around 50% of genes (CpG islands). Recently, the evolutionary dynamics that subdue to the origin and maintenance of CpG islands have been modelled (Cohen et al., 2011). The methylation status of CpG dinucleotides is strictly associated to the regulation of chromatin conformation. CpG islands are usually unmethylated, promoting an open chromatin structure suitable for transcription, and their methylation status is dynamically regulated during development. On the contrary, methylated CpG dinucleotides promote a closed chromatin conformation and are more frequent in the bulk of the genome, in particular in repetitive and intergenic sequences, where they play a central role in maintaining genomic stability and in suppressing spurious transcription.

2.2. Age-associate changes in global DNA methylation

Global DNA methylation level of mammalian genomes decreases with age (Fig. 1, locus A). This observation was firstly established more than three decades ago (Vanyshev et al., 1973, 1970) and was then confirmed in multiple species and tissues, including brain, liver, small intestine mucosa, heart, spleen and blood cells (Drinkwater et al., 1989; Fuke et al., 2004; Golbus et al., 1990; Richardson, 2003; Wilson and Jones, 1983; Wilson et al., 1987). Age-dependent global hypomethylation reflects the decrease in methylation of repetitive tandem and interspersed elements, which are highly enriched in CpG dinucleotides (Jintaridth and Mutirangura, 2010; Rodriguez et al., 2008). Longitudinal models have been exploited to assess intra-individual changes in global DNA methylation over time (Björnsson et al., 2008; Bollati et al., 2009), while the genetic cues that affect the maintenance of epigenetic patterns during ageing have been investigated using family-based cohorts (Björnsson et al., 2008). A recent study considered the relationship between global DNA methylation and frailty, a common clinical syndrome in the elderly (Bellizzi et al., 2012). Interestingly, the authors showed that global DNA methylation levels were lower in 65–85-year-old frail individuals compared to prefrail and nonfrail subjects, suggesting that the relaxation in the epigenetic control of the genome could be associated with the age-associated functional decline.

2.3. Age-associated changes in DNA methylation at gene promoters

Age-dependent DNA methylation changes have been extensively assessed not only at a global level, but also at specific genomic loci. This has allowed the identification of age-associated differentially methylation regions (aDMR) in multiple human tissues. In contrast to global hypomethylation, the trend for gene-associated CpG islands is towards the increase of DNA methylation during ageing (Fig. 1, locus B), strictly resembling what happens in cancer (Gilbert, 2009). The list of promoters whose DNA methylation changes upon ageing is continuously increasing (Table 1), prompted by the advancements in technologies to measure sequence-specific DNA methylation (Ndlovu et al., 2011). Currently, candidate gene approaches are generally performed using the Qiagen PyroMark and Sequenom EpiTYPER platforms, that allow high throughput, highly quantitative analysis of 50–500 nt sequences at single base resolution. On the other side, the most
used techniques for genome-wide analysis of DNA methylation include methylated DNA immunoprecipitation coupled with next-generation DNA sequencing (MeDIP-seq) and methylation microarrays from Illumina (including GoldenGate assay, the Infinium HumanMethylation27 BeadChip and the higher density Infinium HumanMethylation450 BeadChip), while the use whole-genome bisulfite sequencing is less popular due to computational challenges and high costs. Genome-wide approaches have been adopted in a number of age-focused studies, drastically increasing our knowledge of the epigenetic mechanisms of ageing (Table 2).

The first studies of this kind confirmed that age-associated methylation pattern is dependent on genomic context, with a propensity towards hypermethylation of CpG-island loci and towards hypomethylation of non-island CpGs (Christensen et al., 2009). aDMR in whole blood, sorted CD14+ monocytes and sorted CD4+ T-preferentially occur at bivalent chromatin domains (Rakyan et al., 2010), i.e. developmental gene promoters that in stem cells show both marks of active and inactive chromatin and that are often hypermethylated in cancer. These observations are consistent with the idea that ageing and cancer share common defects in cell differentiation and development pathways and account for the higher prevalence of cancer in the elder population. Interestingly, bivalent chromatin domains were recently identified as preferential sites of age-associated hypermethylation also in the

Table 1
Gene-specific epigenetic changes associated with ageing.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue</th>
<th>Methylation change with ageing</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCR, INOS, TLR2</td>
<td>Whole blood</td>
<td>Hypomethylation</td>
<td>Madrigano et al. (2012)</td>
</tr>
<tr>
<td>IFNγ, F3, CRAT, OGG</td>
<td>Whole blood</td>
<td>Hypomethylation</td>
<td>Madrigano et al. (2012)</td>
</tr>
<tr>
<td>EDARAADD, TOM1L1, NPTX2</td>
<td>Whole blood</td>
<td>Hypomethylation</td>
<td>Becklant et al. (2011) and Hannum et al. (2013)</td>
</tr>
<tr>
<td>ELO2</td>
<td>Whole blood, breast, kidney, lung</td>
<td>Hypomethylation</td>
<td>Garagnani et al. (2012) and Hannum et al. (2013)</td>
</tr>
<tr>
<td>Lox, p18INK4a, RUNX3, TIG1</td>
<td>Gastric mucosa</td>
<td>Hypermethylation</td>
<td>Choi et al. (1996)</td>
</tr>
<tr>
<td>c-fos</td>
<td>Liver</td>
<td>Hypermethylation</td>
<td>Ahuja et al. (1998)</td>
</tr>
<tr>
<td>SFRP1</td>
<td>Human colon</td>
<td>Hypermethylation</td>
<td>Issa et al. (1994)</td>
</tr>
<tr>
<td>IGF2</td>
<td>Human colon</td>
<td>Hypermethylation</td>
<td>Issa et al. (1996)</td>
</tr>
<tr>
<td>MYOD1, N33</td>
<td>Human colon</td>
<td>Hypermethylation</td>
<td>Wallace et al. (2010)</td>
</tr>
<tr>
<td>ER</td>
<td>Human colon</td>
<td>Hypermethylation</td>
<td>Issa et al. (1996)</td>
</tr>
<tr>
<td>Myb</td>
<td>Liver</td>
<td>Hypermethylation</td>
<td>So et al. (2006)</td>
</tr>
</tbody>
</table>

Fig. 1. Age-associated changes in DNA methylation patterns. The figure reports the methylation pattern of 3 genomic loci, indicated as A, B and C, in 4 cells from the same tissue in a young (left) and an old (right) subject. In each DNA molecule, a CpG site can be unmethylated (white circle) or methylated (black circle); for clarity, the methylation profile of only one of the two homologous chromosomes is reported. In the bottom of the figure, the DNA methylation levels measured by current approaches are reported. Note that, as the current methods to analyze DNA methylation do not have single-cell resolution, they provide an estimate of the mean methylation level of each site in the cellular population. Repetitive elements (locus A) tend to become unmethylated during ageing, leading to a decrease in the global DNA methylation levels of the genome. The CpG islands at the promoter of some genes (locus B) are hypermethylated in elderly respect to young subjects as a consequence of an “ageing program” or of age-associated environmental cues. Finally, the maintenance of the methylation patterns at some genomic loci (locus C) is impaired in the elderly, leading to increased cell-to-cell heterogeneity in DNA methylation profiles. As a consequence, the methylation levels measured by current approaches tends towards 50%.
human brain (Numata et al., 2012; Watson et al., 2012), suggesting a process common to multiple tissue types.

Two studies compared DNA methylation patterns of centenarians and young subjects (Gentilini et al., 2012; Heyn et al., 2012) and confirmed that methylome encounters drastic changes during ageing. Interestingly, both studies highlighted that age-associated DNA hypermethylation preferentially occurs at gene promoters involved in the regulation of developmental patterns. An increasing interest is directed towards the identification of epigenetic biomarkers that could predict the rate of ageing of an individual and possibly discern between chronological and biological age of the subject. Bocklandt and coworkers demonstrated that methylation of the promoters of EDARADD, TOM1L1 and NPTX2 genes linearly changes with age in saliva and that it can be used to predict age (Bocklandt et al., 2011). Garagnani et al. (2012) analyzed whole blood DNA methylation in 64 subjects from 0 to 99 years using the Infinium HumanMethylation450 BeadChip and identified the promoters of 3 genes, ELOVL2, FHL2 and PENK, whose DNA methylation strongly correlates with age. Validation in a larger cohort of 500 subjects aged 0–99 confirmed a very high correlation with age for the 3 loci and showed that ELOVL2 methylation progressively increases from 0% in newborns to almost 100% in nonagenarians. This result is unlikely to be ascribed to the alterations in blood cellular composition that occur with ageing, but it is probably more linked to epigenetic modifications that arise in progenitor stem cells, as previously suggested (Rakyan et al., 2010). ELOVL2 was included in a quantitative model of ageing based on Infinium HumanMethylation450 BeadChip results on whole blood samples from 656 subjects from 19 to 101 years old (Hannum et al., 2013). This model demonstrated high accuracy in predicting individual’s age and accounted for relevant factors in ageing, such as gender and genetic variants. Moreover, the authors reported that the model was successful in predicting chronological age also in tissues other than whole blood, including breast, kidney and lung.

This last observation prompts further considerations regarding the role of epigenetic modifications in tissue homeostasis and ageing. Indeed, ageing is a multi-factorial process in which, as conceptualized by the “mosaic of ageing” theory, different organs and tissues age at different rate and differently contribute to systemic ageing process (Cevenini et al., 2008). In this framework, although the identification of epigenetic changes that are consistent across different tissues (Christensen et al., 2009; Hannum et al., 2013; Koch and Wagner, 2011) can shed light on the general chromatin remodelling that occurs with ageing, equal emphasis should be placed in the discovery of tissue-specific epigenetic alterations. Consistently with these considerations, Hannum and colleagues reported that the entity of variation of the CpG sites included in the model differed among tissues, suggesting that epigenetic variations can reflect different ageing rates/mechanisms of different tissues (Hannum et al., 2013). In addition, when quantitative models of ageing were built independently for each available tissue, most of the markers were different, confirming tissue-specific patterns of epigenetic changes. Notably, ELOVL2 was included in each tissue-specific model, suggesting that its methylation is uniformly affected by ageing in different tissues and thus highlighting its potential role as biomarker at the organism level.

### 2.4. Triggers and consequences of DNA methylation changes during ageing

In the previous paragraph we have reported several examples of age-associated changes which are systemic, that is they involve the same genomic region in a proportion of the cellular population large enough to be experimentally detectable (Gravina and Vijg, 2010). Some of the systemic changes arise in response to ageing-associated environmental factors, while others are interpreted as part of an “ageing program” which is part of a development (De Magalhães, 2012). Independently of the causes of these systemic changes, current knowledge suggests that global and locus-specific DNA methylation levels are promising markers of human ageing (Johnson, 2006) that can describe the inter-individual variability in functional decline and vulnerability to diseases in the elderly (Bellizzi et al., 2012; Horvath, 2013).

Together with systemic changes, the epigenome is also subjected to stochastic changes, i.e. random variations in the epigenetic patterns that have been called epimutations (Holliday, 1987; for an exhaustive review, see Gravina and Vijg, 2010). During ageing a relaxation of the mechanisms that maintain the epigenetic patterns occurs, leading to increased cell-to-cell heterogeneity in both gene expression (Bahr et al., 2006) and epigenetic markers (Fig. 1, locus C) (Bennett-Baker et al., 2003; Fu et al., 2008). Epimutations can contribute to the age-associated
epigenetic drift, which consists in the progressive divergence of the epigenetic patterns between individuals with time. In a seminal paper, Fraga and colleagues showed that the methylymes of young twin couples are more similar than those of elderly couples (Fraga et al., 2005). Based on these observations, several studies have analyzed the age-associated divergence in DNA methylation patterns between twins (Heijmans et al., 2007; Pirazzini et al., 2012; Talens et al., 2012) and have shown that (i) some genomic loci are epigenetically more stable than others; (ii) the ability to maintain DNA methylation patterns differs among different individuals and it is genetically determined, as suggested also by longitudinal family-based models (Bjornsson et al., 2008).

Regarding this epigenetic drift, it is difficult to discern the relative contributes of environmental-driven and stochastic changes in DNA methylation, but recent experiments using laboratory animals, whose environmental conditions can be strictly monitored, have shown that chromatin entropy increases with age, while chromatin homogeneity decreases, pointing for a key role of epimutations in the ageing process (Pantic et al., 2012).

Age-associated changes in DNA methylation are triggered, at least in part, by alterations in the expression/activity of DNA methyltransferases (DNMTs). Indeed, several authors reported that a decrease in Dnmt1 and Dnmt3a expression occurs with ageing, while the levels of Dnmt3b follow the opposite trend (Casillas et al., 2003; Liu et al., 2009; Lopatina et al., 2002). Accordingly, while Dnmt1 knock-down promotes age-related phenotypes (Liu et al., 2011; Ray et al., 2006), the rescue of Dnmt3a2 levels restores cognitive function in aged mice (Oliveira et al., 2012). Beside DNMTs expression, many other mechanisms can affect the maintenance of DNA methylation during ageing. For example, other epigenetic modifications and proteins involved in the DNA damage response can modulate the access of DNMTs to target sequences and affect the methylation status of CpG dinucleotides (Ciccàrone et al., 2012; Guastaferro et al., 2008; Jin and Robertson, 2013).

Future researches should address not only the causes of DNA methylation changes during ageing, but also their consequences. While the contribution of DNA methylation changes at promoters should be evaluated on the basis of the function of the specific gene, the global hypomethylation of the genome could exert ageing-effects by promoting genomic instability and activation of transposable elements (Alexeeff et al., 2013; Barbot et al., 2002; De Cecco et al., 2013).

3. Epigenetic mechanisms in anti-ageing nutritional interventions

Epigenetic patterns are set during gametogenesis, fertilization and in utero development and they are particularly responsive to environmental factors in these periods. Accordingly, a number of studies both on murine and human models have highlighted the epigenetic consequences of nutritional interventions during preconceptional and in utero periods and their transgenerational heritability. These studies have been deeply reviewed in recent manuscripts (Barnes and Ozanne, 2011; Gabory et al., 2011; Lillycrop and Burdge, 2012) and will not be reported here. On the contrary, we will specifically focus on the epigenetic plasticity in the adulthood, reporting available evidences that diet interventions can alter epigenetic patterns later in life and positively affect ageing and lifespan. The example of honeybees is a proof of principle of this deep relationship between after birth diet, epigenetics and ageing, as female larve develop in short-living workers or in long-living queens according to DNA methylation modifications induced by the type of feeding (i.e. beebread or royal jelly) (Kucharski et al., 2008). Examples of diet-mediated changes in epigenetic modifications have been reported also in humans.

![Fig. 2. Nutritional interventions, including caloric/dietary restriction, nutrients involved in one-carbon metabolism and bioactive food components, can modify DNA methylation and/or other epigenetic modifications, that in turn can affect DNA methylation patterns.](http://dx.doi.org/10.1016/j.mad.2013.12.006)
The extent of age-related variations in leukocytes global DNA methylation was associated with the daily intake of carbohydrates, lipids, vitamin B6 and magnesium (Gomes et al., 2012). A recent study considered the effect of a diet rich in vegetables and plant oil containing polyunsaturated fatty acids on the methylation of MLH1, a gene involved in mismatch repair and in maintenance of genomic integrity (Switzeny et al., 2012). The intervention resulted in significant increase in MLH1 CpG methylation that was not associated to variations in its expression but that probably reflected a decrease in reactive oxygen species (ROS) and in the demethylating effects of DNA damage related enzymes.

In the following paragraphs we will summarize the effects of dietary restriction and different bioactive compounds on DNA methylation changes during ageing (Fig. 2).

3.1. Caloric and dietary restriction

Although the notion that limited food intake can positively affect health and ageing has deep roots in history, the first scientific demonstration that reducing food intake significantly increases rodents life span dates back to 1935 (McCay et al., 1989). Since then, a number of dietary modifications have been experimented. Caloric restriction (CR) consists in the reduction of caloric intake, typically by the 20–40% respect to the ad libitum intake of the species, without leading to malnutrition and deficiency in micronutrients. CR is distinct from dietary restriction (DR), in which one or more macronutrients is reduced or removed without affecting the total caloric intake, and from regular fasting such as alternate-day fasting (ADF) (Trepanowski and Bloomer, 2010).

The effects of these dietary modifications on health and longevity have been investigated in many species, from yeast to primates, leading to exciting but sometimes controversial results (Speakman and Mitchell, 2011). For example CR has proven to extend lifespan in many animal models and to effectively protect against age-related diseases such as diabetes and cancer in murine models. However, some evidences indicate that these outcomes are not universal, and that factors such as genetic background (Liao et al., 2010) and initial basal metabolic rate (Brzek et al., 2012) can affect this phenomenon. The results from two long-term studies on the effects of CR in non-human primates have been recently published (Colman et al., 2009; Mattison et al., 2012). Both studies reported health benefits such as reduction of the incidence of diabetes and of cancer in calorie restricted rhesus monkeys respect to controls, but were discordant on the effects of CR on life span extension. These apparently conflicting results probably arise from the design of the two studies that differ for the diet composition of both caloric restricted and control monkeys. Data on the effects of CR on human models are also available. For example, a randomized clinical study on non-obese, sedentary humans, demonstrated that a 20% reduction in calorie intake for 12 months decreases visceral fat mass, improves insulin sensitivity, increases plasma adiponectin concentration, reduces circulating inflammatory markers, decreases plasma triiodothyronine levels, and reverses some of the age-related deterioration in cardiac diastolic function (Fontana et al., 2010a; Stein et al., 2012; Weiss et al., 2008). Ongoing studies on human subjects who voluntarily follow a caloric restricted diet will contribute to clarify not only the potential anti-ageing effects of CR (Mercken et al., 2013), but also its contribution to the slowdown of age-associated diseases onset (Stein et al., 2012).

The molecular mechanisms by which CR exerts its beneficial effects are poorly understood, but two nutrient-sensing signalling networks, the insulin/IGF (insulin-like growth factor) and the mTOR (mammalian target of rapamycin) pathways, seem to respond to CR in the control of cell growth and ageing pathways (Speakman and Mitchell, 2011). In addition, some authors have suggested the involvement of Sirtuins, a group of NAD-dependent histone acetylase, but their role in linking CR to longevity is, at least in mammals, still controversial (Baur et al., 2010; Fontana et al., 2010b). It is worth to note that both mTOR (Grummt and Voit, 2010; Murayama et al., 2008) and Sirt-1 (Salminen and Kaarniranta, 2009) link the cellular energy condition with the epigenetic status of ribosomal DNA, which in turn plays a role in longevity in model organisms (Larson et al., 2012).

Paradoxically, the possibility that CR could exert anti-ageing effects by reversing epigenetic alterations during ageing and age-related diseases has been relatively poorly investigated (Li et al., 2011b; Vaquero and Reinerberg, 2009). In this sense, it is relevant the recent study by Ions et al. (2012) who analyzed available public databases and reported a significant overlap between the genes differentially expressed in response to CR and those whose methylation varies during ageing. Two independent studies reported that CR increases methylation levels of MYC in mice liver (Miyamura et al., 1993) and of HRAS in pancreatic acinar cells (Hass et al., 1993), reverting the trend towards age-associated hypomethylation of the two oncogenes. More recently, CR was shown to counteract the increase in Dnmt3a expression in the hippocampal dentate gyrus that is observed in aged mice (Choularas et al., 2011) and to prevent the age-related changes in DNA methylation levels (Choularas et al., 2012). In an in vitro model of CR, glucose restriction increased Dnmt1 activity and induced hypermethylation of a E2F-1 binding site in the promoter of the CDKN2A gene, down-regulating its expression and extending cell lifespan (Li et al., 2010a,b). As the tumor suppressor p16 accumulates during senescence, its decreased expression could contribute to CR-mediated longevity in vivo. The same group reported that p16 reduction upon glucose restriction was ascribable to the combined effect of DNA methylation and Sirt-1 mediated histone decetylation, supporting a link between these two chromatin modifications (Li et al., 2011b). In line with these evidences, manipulation of Sirt-1 levels by overexpression or silencing of the gene in Caco-2 cells altered the methylation of six genes whose expression is responsive to CR (Ions et al., 2012). Epigenetic data on the effects of CR in humans is limited. In a cohort of overweight/obese postmenopausal women before and after a reduced energy intake intervention over 6 months, epigenomic analysis in subcutaneous adipose tissue showed significant hypermethylation of three loci (chromosomes 1p36, 4q21, and 5q13) in responders to the treatment (Bouchard et al., 2010). In a similar study, two CpG sites in the APT10A and in the WT1 genes were hypermethylated in peripheral blood mononuclear cells (PBMCs) in overweight/obese men after 8 weeks dietary intervention (Milagro et al., 2011). As obesity is a major risk factor for age-related diseases, these studies can shed light on the mechanisms by which CR can potentially exert its anti-ageing effects.

As CR is not a feasible nutritional intervention in the general population, researchers have looked for mimetics of CR that do not imply food restriction (Riba, 2012). Protein restriction, and in particular methionine restriction, seems to be an effective alternative to CR in lifespan extension (Miller et al., 2005). Methionine is the precursor of S-adenosylmethionine (AdoMet) in one-carbon metabolism (see below), but the outcomes of its restriction on DNA methylation have been considered only in few studies. For example, 7 weeks 40% methionine restriction induced DNA hypomethylation in rat heart (Sanchez-Roman et al., 2011) but not in rat liver (Sanchez-Roman et al., 2012), suggesting a tissue-specific effect. Two other potential CR mimetics are rapamycin, a bacterial macrolide with antifungal and immuno-suppressant activities inhibiting mTOR, and resveratrol, a polyphenol (see below) enriched in the skins of red grapes that can activate Sirt-1 (Barger et al., 2008). However, also in this case the epigenetic mechanisms by which they can exert an anti-ageing...
effect have not been assessed yet. These compounds, together with other bioactive food components such as green tea extracts (see below), are being tested by the Interventions Testing Program, a multi-institutional program promoted by the National Institute of Ageing that aims at identify treatments with an anti-ageing effect in mice.

3.2. Nutrients involved in one-carbon metabolism

The one-carbon metabolism is a complex biochemical pathway, also known as the homocysteine cycle (Fig. 1). One of the intermediates of this metabolism is AdoMet, which represents the unique methyl donor in all the DNMTs-mediated DNA methyltransferase reactions in eukaryotes. Various nutrients can regulate the one-carbon metabolism, acting both as sources of methyl donors (for example methionine, choline, betaine and serine) and as co-enzymes (for example vitamins B2, B6, B12 and folate) (Kim et al., 2009). Although the role of these nutrients in physiological and pathological processes has not been completely studied, many evidences support that they can affect DNA methylation changes during ageing.

With the term “folate” we indicate a group of water-soluble B vitamins, also known as B9, that carry a methyl group which can be used for the AdoMet synthesis. As folate and its derivatives directly affect reproductive success and survival early in life, natural selection has worked to protect folate levels and preserve the integrity of folate metabolism. Humans cannot synthesize folate, which therefore has to be supplied through the diet. Folate-rich foods include dark green leafy vegetables, asparagus, broccoli, strawberries, liver, organ meats and legumes. In addition, folate can be supplemented with diet and fortified foods in the form of the synthetic molecule folic acid. Many studies have addressed the relationship between DNA methylation and folate intake during pregnancy (Ciappio et al., 2011; Kim et al., 2009) and cancer (Stefanska et al., 2012), while the effects of folate administration on ageing are less well characterized. Age associated decrease of DNA methylation in mouse colon was reduced by folate administration, which induced also CDKN2A hypermethylation (Keyes et al., 2007). These results are in agreement with those recently achieved by Li and colleagues (Li et al., 2010a,b). In this study, T lymphocytes from healthy subjects aged 22–81 were cultured in a folate-depleted medium. T cells from 50 years or older donors showed hypomethylation of genes that are activated during ageing, such as KIR and CD70 genes, suggesting a synergic effect between age-associated DNA methylation changes and folate levels. A number of studies confirm that a folate-poor diet is associated to global DNA hypomethylation and to an increased risk for chronic diseases (Axume et al., 2007; Pufulete et al., 2005; Rampersaud et al., 2000; Shelnut et al., 2004). On the other side, folate supplementation increased global DNA methylation levels in rectal mucosa of a subset of patients with colonic adenomas (Cravo et al., 1998b) and reduced microsatellite instability in the mucosa of ulcerative colitis patients (Cravo et al., 1998a). The effect of folic acid administration on site specific DNA methylation was analyzed in 1000 colorectal mucosa biopsies (Wallace et al., 2010). In this study estrogen receptor gene α (ESR1) and SRPR1 methylation levels, that increase with age and may predispose to colorectal cancer, were positively correlated with folate levels in red blood cells, posing safety concerns regarding folic acid administration in healthy adults.

Beside folate, other intermediates of one-carbon metabolism can affect DNA methylation and ageing. Vitamin B12 (cyanocobalamin) is a water-soluble vitamin that acts as co-enzyme of methionine synthase. Low vitamin B12 concentration reduces methionine synthase activity and prevent folate metabolism, resulting in a decrease in AdoMet concentration and therefore in DNA methylation. Vitamin B-rich foods include eggs, cheese, beef, lamb, seafood and milk. Deficiencies in vitamin B12 can lead to elevated DNA damage and altered DNA methylation (Blount et al., 1997; Lindahl and Wood, 1999) and to an increase of homocysteine level, a risk factor for cardiovascular disease in particular for the elderly (Krishna et al., 2013; Van Dijk et al., 2013).

It should be noted that DNA methylation responses to B vitamin levels may depend on various factors, including genetic background (Axume et al., 2007; Friso et al., 2002; Shelnut et al., 2004), extent and duration of the depletion/supplementation and the tissues under investigation. For example, Hubner et al. recently reported that one-year vitamin B supplementation did not affect AdoMet plasma levels and long interspersed element-1 (LINE-1) methylation levels in blood cells (Hubner et al., 2013).

3.3. Bioactive food components

Polyphenols constitute a heterogeneous group of natural substances, chemically formed by several phenolic rings, which are particularly known for their positive effects on human health and are enriched in many plant foods. The intake of polyphenols in the human diet varies greatly depending on the type, the quantity, the quality of vegetables consumed and the chosen cooking method that can reduce considerably the polyphenolic content of the food. Polyphenols can exploit a wide range of activities, including antioxidant, antiatherogenic, anticarcinogenic and anti-inflammatory effects. Their antioxidant properties are recognized worldwide: polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of degenerative diseases associated to oxidative damage that can be related to both normal cellular metabolism and stressful events, such as radiation, smoke, pollutants, UV rays, emotional and physical stress, chemical additives, viral and bacterial attacks. Numerous studies have shown that, when added to the diet, polyphenols limit the development of cancers (Darvesh and Bishaye, 2013; Gokbulut et al., 2013; Gollucu et al., 2013; Tabrez et al., 2013), cardiovascular diseases (Kuruma et al., 2013; Li and Förstermann, 2012), neurodegenerative diseases (Cimini et al., 2013), diabetes (De Bock et al., 2013; Patel et al., 2012), obesity (Siriwardhana et al., 2013; Tian et al., 2013) and osteoporosis (Oka et al., 2012; Sacco et al., 2013).

Regarding the effects of polyphenols on DNA methylation, studies have been focused on different pathologies, including cancer (Vanden Berghe, 2012). Several mechanisms by which polyphenols concur to cancer inhibition have been reported. These mechanisms include the reduction of hypermethylation status that characterizes genes such as CDKN2A and RARB. Polyphenols may act both directly through physical interaction with Dnmt1 catalytic site and indirectly by the reduction of intracellular concentration of AdoMet, that in turn reduces Dnmt1 activity (Fang et al., 2007; Li and Tollefsbol, 2010).

Lee et al. (2005) evaluated the effects of several tea catechins and bioflavonoids on DNA methylation, finding that each of the tea polyphenols considered [catechin, epicatechin, and (→)epigallocatechin-3-O-gallate (ECGG)] and bioflavonoids (quercetin, fisetin, and myricetin) inhibited Dnmt1-mediated DNA methylation in a concentration-dependent manner. Many other authors observed the inhibition of DNMTs activity as a consequence of different polyphenols diet supplementation (Fu and Kurzrock, 2010; King-Batoo et al., 2008; Meeran et al., 2010), with tea polyphenols and genistein showing the strongest inhibitory effect.

ECGG is a polyphenolic catechin found in green tea with antioxidant properties that can induce epigenetic changes by inhibiting enzymes involved in DNA methylation and by modifying histone acetylation (Nandakumar et al., 2011; Pandey et al., 2010), Wong et al. reported that the diet ECGG intake could inhibit
DNMTs activity and could induce Foxp3 and IL-10 expression in CD4+(+) Jurkat T cells at physiologically relevant concentrations in vitro, showing a role in controlling the development and function of regulatory T cells (Treg). Furthermore, authors found that mice treated with EGC2 in vivo had a significant increase in Treg frequencies, suggesting, on the whole, that EGC2 can induce Foxp3 expression and increase Treg frequency through an epigenetic-based mechanism (Wong et al., 2011). Qin et al. conducted a study on 34 healthy premenopausal women consuming iso-flavones for one menstrual cycle to test if soy isoflavones have dose-related estrogenic and methylation effects, finding that this kind of polyphenols caused significant changes in the methylation levels of two cancer related genes (RARβ and CCND2) in the breast, according to the circulating levels of genistein (Qin et al., 2009).

Although the data collected until now are encouraging, future studies should specifically consider the effects of polyphenols on DNA methylation in an anti-ageing perspective.

4. MicroRNAs and ageing: the inflamma-miRs

During ageing a low-grade systemic inflammation characterized by elevation of circulating acute-phase proteins and proinflammatory cytokines, for instance IL-6 and TNF-α, occurs; a conflict that we have previously designated inflammaging (Franceschi, 2007; Franceschi et al., 2000). Such inflammatory imbalance is associated to frailty and the development and progression of age-related conditions that include cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), neurodegenerative diseases, sarcopenia and cancer (Cevenini et al., 2013; Franceschi, 2007; Vasto et al., 2007).

The role of DNA methylation in inflammaging has not been disentangled yet, but some evidences point for an association between age-related global DNA hypomethylation and increased expression of a number of inflammatory markers (Agrawal et al., 2010; Alexeef et al., 2013; Baccarelli et al., 2010b). Beside DNA methylation, researchers have examined the function of other epigenetic modifications in the establishment of the inflamma-miRNA phenotype, focusing in particular on microRNAs (miRs).

MiRs are a broad class of small, non-coding RNAs that exert a powerful gene regulatory role, acting both as repressor as well as activators, mainly at a post-transcriptional level (Brevig and Esquela-Kerscher, 2010). The primary transcripts of miRs (pri-miRs) are transcribed by RNA polymerase II–III in the nucleus. Pri-miRs are processed by the Drosha/DGCR8 enzyme complex into 70 base pair precursor and then transported into the cytoplasm, where RNase III enzymes, Dicer and Loquacious, process them into about 22-nucleotide miR duplexes with guide and passenger strands. The guide strand functions as a mature miR and is incorporated into an RNA–induced silencing complex (RISC), containing an Argonaute (Ago) protein. MiRs guides RISC to recognize target sequences located mainly in the 3’UTR of mRNAs, which lead to the inhibition of translation or degradation of mRNA (Bartel, 2009). Since the specificity of miR targeting is mediated only by 6–11 nucleotides, a single miR can target hundreds of mRNAs (Inukai and Slack, 2013; Park and Kim, 2013). MiRs have been reported to act through autocrine and/or paracrine mechanisms (Kumarswamy et al., 2011; Raitharju et al., 2011). In addition, circulating miRs can act as hormones, eliciting a systemic response (Wahlgren et al., 2012). Recent studies show that transfer of nucleic acids, including miRs, can be an important means of intercellular communication, occurring both by direct cell-cell contact, for instance via gap junctions, or by cell-contact-independent mechanisms, including release of microvesicles into surrounding tissue (Collino et al., 2010; Hosoda et al., 2011) or the blood stream. Plasma exosomes can deliver exogenous short interfering RNA, including miRs to monocytes and lymphocytes (Wahlgren et al., 2012).

Even if a single miR can target hundreds of mRNAs, groups of miRs can induce regulation of specific biological processes by acting in a co-ordinated manner on pathways of functionally related genes (Cloonan et al., 2011). Thus, miRs have recently been indicated as regulators of a number of cellular processes such as apoptosis, differentiation, cell cycle, and immune functions, and their expression can be altered by a wide spectrum of environmental factors including drugs, virus and bacterial pathogens, cigarette smoking, alcohol, sleep, exercise, stress, radiation and nutrition.

It is worth stressing that exceptionally long survival requires dynamic preservation of optimal levels of physiological variables, and that the mean levels of many biomarkers of ageing are not stable, but change in the course of life (Spazzafumo et al., 2013). Thus, a peculiar modulation of miRs expression might contribute to efficient homeostasis in human ageing (Grillari and Grillari-Voglauer, 2010; Inukai and Slack, 2013). Recently we discussed the pleiotropic effects exerting of some miRs on pathways related to inflammation, senescence, and carcinogenesis (Olivieri et al., 2013a). A number of miRs have been reported to play a role in modulating cellular senescence and inflammatory responses (α-miRs and inflamma-miRs, respectively). Given the interest elicited by this new area of research, more and more miRs involved in the modulation of inflammation and senescence are expected to be identified in the near future. A clear link between miRs and longevity has been demonstrated in C. elegans, suggesting that miRs could have relevant role also in regulation of human lifespan and ageing process (Ibáñez-Ventoso and Driscoll, 2009). However, only few studies have compared the miRs expression profile in tissues and blood of old and young organisms of different species (Table 3). Interestingly, differences in the circulating miRs have been found in a variety of age-associated diseases. The origin of circulating miRs is not completely defined, but senescent cells emerge as a possible source of such secreted miRs, suggesting that these miRs might contribute to the functional decline observed during ageing (Weinler et al., 2012). Overall the results of these studies supported the hypothesis that achievement of extreme longevity probably requires a special gene expression regulation involving inflamma-miRs (Elsharawy et al., 2012; Gombar et al., 2012; Olivieri et al., 2012a; Serna et al., 2012). The prototype of inflamma-miRs are miR-155, miR-21, miR-146a and miR-126 (Olivieri et al., 2013b, 2012b; Quinn and O’Neill, 2011). Under physiological conditions, transcription of the inflamma-miRs is maintained at basal levels; however, as soon as the pro-inflammatory signalling are promoted, their expression is strongly co-induced through a mechanism that is mainly NF-κB-dependent (Baldin and Baltimore, 2012; Olivieri et al., 2013b). Although the importance of inflamma-miRs in the regulation of the innate immune response and the acquisition of senescence-associated secretory phenotype during cellular senescence is clearly demonstrated, the molecular mechanism of their action turned out to be much more complex than initially thought (Olivieri et al., 2012a; Grillari and Grillari-Voglauer, 2010).

5. Epigenetic diets and inflamma-miRs

A growing number of evidences support the role of diet in slow down and at least in part revert inflamma-miRs (Proietti et al., 2009). As diet is known to influence the expression of miRs, nutrients with antioxidant and anti-inflammatory effects were investigated to verify their ability to modulate the expression of specific miRs among which inflamma-miRs. A potential role of miR-126 in the anti-inflammatory properties of polyphenols from red wine was observed in human colon myofibroblasts cells (CCD-18Co).
Table 3
Main miRs associated with organismal ageing.

<table>
<thead>
<tr>
<th>MiRs</th>
<th>Organism</th>
<th>Tissue</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-20a, miR-106a, miR-126, miR-155</td>
<td>Humans</td>
<td>Whole blood</td>
<td>ElSharawy et al. (2012)</td>
</tr>
<tr>
<td>miR-21</td>
<td>Humans</td>
<td>Plasma</td>
<td>Olivieri et al. (2012b)</td>
</tr>
<tr>
<td>miR-126</td>
<td>Humans</td>
<td>Plasma</td>
<td>Our unpublished data</td>
</tr>
<tr>
<td>miR-21</td>
<td>Humans</td>
<td>PBMCs</td>
<td>Serna et al. (2012)</td>
</tr>
<tr>
<td>miR-21</td>
<td>Humans</td>
<td>B lymphocytes</td>
<td>Gombart et al. (2012)</td>
</tr>
<tr>
<td>45 miRs</td>
<td>Male mice</td>
<td>Serum</td>
<td>Dhabhi et al. (2013)</td>
</tr>
<tr>
<td>miR-34a</td>
<td>Mice</td>
<td>Plasma, PBMCs, brain</td>
<td>Li et al. (2011a)</td>
</tr>
<tr>
<td>93 miRs</td>
<td>Mice</td>
<td>Brains</td>
<td>Innai et al. (2012)</td>
</tr>
<tr>
<td>miR-34a, miR-93, miR-214, miR-669c, miR-709</td>
<td>Mice</td>
<td>Liver</td>
<td>Smith-Vikos and Slack (2012)</td>
</tr>
<tr>
<td>miR-22, miR-101a, miR-720, miR-721</td>
<td>Mice</td>
<td>Brain</td>
<td>Smith-Vikos and Slack (2012)</td>
</tr>
<tr>
<td>miR-7, miR-468, miR-542, miR-698, miR-124a, miR-181a, miR-221, miR-382, miR-434, miR-455</td>
<td>Mice</td>
<td>Skeletal muscle</td>
<td>Smith-Vikos and Slack (2012)</td>
</tr>
<tr>
<td>65 miRs, including miR-21</td>
<td>Mice</td>
<td>Heart</td>
<td>Zhang et al. (2012)</td>
</tr>
<tr>
<td>let-7 miR cluster</td>
<td>Mice</td>
<td>Heart</td>
<td>Cao et al. (2012)</td>
</tr>
<tr>
<td>miR-34</td>
<td>Drosophila</td>
<td>Brain</td>
<td>Liu et al. (2012)</td>
</tr>
<tr>
<td>miR-124</td>
<td>Different species</td>
<td>Different tissues</td>
<td>Dallaire et al. (2012)</td>
</tr>
</tbody>
</table>

(Angel-Morales et al., 2012). MiR-126 targets NF-κB signalling (Olivieri et al., 2013b) and miRNA levels of adhesion molecules, such as Icam-1, Vcam-1, and Pecam-1.

The anti-inflammatory properties of the flavonol quercetin have been intensively investigated using in vitro cell systems and are to a great extent reflected by changes in the expression of inflammatory markers (Boesch-Saadatmandi et al., 2012). Quercetin protected CCD-18Co myofibroblasts against ROS in part by increasing activity of antioxidant enzymes and in part by inducing an up-regulation of miR-146a, known as a negative regulator of pro-inflammatory NF-κB activation, thus protecting CCD-18Co from inflammation (Noratto et al., 2011). Moreover, hepatic miR-122 and miR-125b concentrations, previously involved in inflammation (miR-125b) and lipid metabolism (miR-122), were increased by dietary quercetin supplementation, suggesting that miRs modulation can contribute to the gene-regulatory activity of quercetin in vivo (Boesch-Saadatmandi et al., 2012). Resveratrol and quercetin in combination are able to decrease the generation of ROS in colon cancer cells decreasing in turn oncogenic miR-27a expression (Del Follo-Martinez et al., 2013).

Interestingly, the stimulation of specific signalling pathways can occur in the cross-talk between probiotic bacteria and gut epithelium cells, which can help to explain the adjuvant properties of probiotic lactobacilli (Vizoso Pinto et al., 2009). Strain specific properties of probiotics in providing supportive health effects in the immune system and the gastrointestinal tract have been widely investigated in vivo and in vitro, suggesting that specific strains could even modify the immune response at post-transcriptional level by modifying miRs expression in dendritic cells (DCs) (Gaihi et al., 2012). Probiotic strains can affect toll like receptor 4 (TLR4) expression in a down-regulatory direction, reducing in turns inflammation level. Inactivated strains of Lactobacillus rhamnosus GG (LGG) in human DCs can induce a significant down-regulation of miR-146a expression and a concomitant up-regulation of miR-155 in DCs, which is consistent with the down-regulation of p38MAPK (Gaihi et al., 2012). It was reported that these two seemingly co-induced regulatory RNAs, miR-146a and miR-155, dramatically differ in their induction behaviour under different stimuli strengths and act non-redundantly through functional specialization.

In conclusion, inflammation-miRs seem to be modulated by nutrients, highlighting the potential role of probiotic intervention in reducing the pro-inflammatory status associated with human ageing.

6. A notable example: cardiovascular disease

In the developed countries most of the elderly are affected by age-related chronic diseases, which share an inflammatory background. These pathologies share features of accelerated ageing, which from an epigenetic point of view consist in global hypomethylation and site-specific hypermethylation of the genome as discussed above. The ongoing epigenetic characterization of age-related diseases has two important consequences: (1) allows the identification of epigenetic markers of biological age that can be used to early detect the pathologies; (2) provides an interventional target. Indeed, most of the age-related diseases are multi-factorial pathologies in which environmental lifestyle, and in particular nutritional habits, plays a pivotal role. Based on these considerations, it is clear that the anti-ageing dietary interventions provide a powerful tool to counteract or slowdown the age-related diseases onset. In this paragraph, we discuss cardiovascular diseases as a notable example.

Recent studies reported the function of epigenetic mechanisms in CVDs (Baccarelli et al., 2010a; Handy et al., 2011; Turunen et al., 2009; Udali et al., 2013). Epigenetic modification can regulate gene expression of key pathways related to coronary artery disease development in human and animal models (Baccarelli et al., 2010a; Creemers et al., 2012; Hiltunen et al., 2002a,b; Laukkonen et al., 1999) or specifically affect the expression of genes involved in its major thrombotic complication, myocardial infarction (Friso et al., 2012; Handy et al., 2011). Changes in both genome and site-specific DNA methylation have been described in CVD (Friso et al., 2012, 2008) although their specific function is still to be largely investigated (Baccarelli et al., 2010a).

Hiltunen et al. (2002b) described the first report of a global hypomethylation, in advanced atherosclerotic lesions in humans as well as mouse and rabbit models. However, whether global DNA hypomethylation has a causal role or is it a consequence of atherogenesis is not yet known. Global DNA hypomethylation was described also in atherosclerosis-prone apoE−/− mice compared to C57BL/6 controls at early disease stages leading to the hypothesis that aberrant DNA methylation may precede the onset of disease even of several years, as described for cancer disease (Ehrlich, 2006; Lund et al., 2004).

Some studies analyzed the levels of global DNA methylation in association with plasma concentrations of homocysteine (Hcy), a biomarker for vascular disease (Handy et al., 2011). Castro et al. (2003) described a significant decrease in DNA global methylation in white blood cells DNA of patients with vascular disease and higher Hcy, compared to a control group. The higher plasma Hcy paralleled with increased concentrations of S-adenosylhomocysteine (AdoHcy) (Castro et al., 2003), the intracellular precursor of Hcy with a well-known function as a strong inhibitor of DNMTs (Vliet et al., 2000). Considering that both Hcy (Girelli et al., 1998; Jacques et al., 1996) and DNA methylation are dependent on folate concentrations through a gene-nutrient interaction model
(Friso et al., 2013, 2002) it seems of high interest to explore whether nutritional regulation within one-carbon metabolism may modify the risk of CVD through DNA methylation. A recent observation reported that a nutrient-gene interaction within folate metabolism, not only affect the levels of global DNA methylation but also the risk of cancer disease through this epigenetic phenomenon (Friso et al., 2013). Whether this finding may also apply to CVD is still to be evaluated. Using a surrogate marker for global DNA methylation, LINE-1 methylation was evaluated as a possible marker for cardiovascular risk (Baccarelli et al., 2010c). Different results have been reported, however, when global methylation in peripheral blood leukocytes was evaluated by measuring Alu and juxta-centromeric Satellite 2 repetitive elements (Kim et al., 2010) whose methylation highly correlate with global methylation of DNA (Weisenberger et al., 2005). The precise reason of the inconsistencies observed in those studies may be found in either the different study design but also in the techniques utilized for measuring DNA methylation, namely bisulfite-treatment-based methods (Baccarelli et al., 2010c; Kim et al., 2010), restriction enzyme digestion (Castro et al., 2003; Lund et al., 2004; Yi et al., 2000) or HPLC-based methods (Hiltunen et al., 2002b) which may indeed account for the results variability.

Several studies reported the role of methylation at specific gene-sites in CVD. One of the first report refers to the epigenetic regulation through methylation of the estrogen receptors α (ESR1) and β (ESR2) genes whose functions have been largely described for CVD (Mendelsohn and Karas, 1999). Post and colleagues observed higher methylation levels of ESR1 promoter region in human coronary atherosclerotic plaques compared to normal vascular tissues (Post et al., 1999). The ESR2 methylation at promoter region was also described in human atherosclerotic lesions where the increase in DNA methylation correlated with decreased ESR2 expression (Kim et al., 2007).

Gene-specific DNA methylation in human atherosclerotic lesions was also studied by analyzing the gene coding for 15-lipoxygenase (ALOX15), a lipid peroxidating enzyme strongly expressed in atherosclerotic plaque (Hiltunen et al., 2002b). Using a bisulfite-sequencing approach a hypomethylation in CpG promoter region sequences was observed that possibly account for the increased ALOX15 gene expression.

The role of methylation in CVD was also studied at coagulation factor 7 gene (F7) promoter site considering that factor VII plasma concentrations are a strong risk factor for coronary artery disease. F7 promoter was evaluated in the DNA extracted from PBMCs of patients affected by coronary artery disease (CAD) compared to CAD-free subjects where F7 promoter hypomethylation correlated with higher plasma concentrations of FVIIa and higher risk for CAD, therefore highlighting a possible role for methylation at F7 promoter site and CAD risk (Friso et al., 2012).

Regarding the inter-relationships among nutritional factors, DNA methylation and CVD risk, van Straten and colleagues recently showed that a low protein diet influences adult lipid metabolism through DNA methylation by altering, among several differentially methylated genes, liver X-receptor alpha (NR1H3) (Van Straten et al., 2010). This gene is involved in the control of cholesterol and fatty acid metabolism and it is hypothesized to contribute to the risk of CVD.

A very interesting model is that proposed by Lillycrop and colleagues who showed, using a rodent model, that a protein restricted diet induces a lower promoter methylation status in glucocorticoid receptor (NR3C1) and peroxisomal proliferator-activated receptor α (PPARα): both genes are related to cardiovascular disease also in humans by being involved in the regulation of blood pressure and in the lipid and carbohydrate homeostasis, respectively (Lillycrop et al., 2008, 2005).

A growing interest is emerging for the role of miRs and CVD mostly due to the hypothesis of being potentially useful biomarkers of disease development or progression (Bauersachs and Thum, 2011). In this view, miRs have been largely explored in both plasma and serum of stable CAD patients mostly to evaluate whether miRs usually expressed in endothelial cells (miR-126, miR-17, miR-92a), in smooth muscle cells (miR-145) or in inflammatory response-associated cells (miR-155) may also serve as possible CVD markers (Fichtlscherer et al., 2010). However, whether specific miRs plasma levels will be useful markers of disease diagnosis or complication in the near future is not clearly known. Moreover, it is still to be explored whether miRs profiles may be modifiable by environmental or nutritional factors as occurring to other epigenetic features of DNA. The latter issue is of great importance considering that by modulating the environment or nutritional setting it could be possible to modify disease risk or adverse progression.

7. Conclusions

Unravelling the molecular basis of successful ageing is a challenging but exciting goal of current research. Epigenetics is gaining a central position in this scenario, as it provides at the same time mechanistic insights into the ageing process and scientific basis for interventional strategies. A growing number of studies demonstrate that age-associated epigenetic variations can be positively affected by physical exercise, lifestyle habits and diet. However, it should be kept in mind that we are still at the beginning of such studies, and that much has to be done in order to disentangle the complex relationship between epigenetics, nutrition and ageing. For example, the tissue (or even cellular) specificity of age-associated epigenetic changes and their functional contribution to ageing deserve deeper investigation, together with the molecular mechanisms by which dietary habits and food components affect the epigenetic patterns. Among all tissues, adipose tissue appears to be critical for the tight connections between over-nutrition and inflammatory status, being a paradigmatic example of the powerful effects of the underlying epigenetic changes (see the paper by Zamboni et al., 2013, in this special issue). In addition, the stability of the epigenetic modifications induced by nutritional interventions should be evaluated over time. Finally, although the use of murine models is a valuable tool in these studies, the availability of opportune studies on humans, such as those provided by the European Project NU-AGE (see Box 1), appears unavoidable to make in the next future epigenetic diets a valid interventional approach for successful ageing.

Acknowledgements

This study was supported by: the European Union’s Seventh Framework Program under grant agreement n° 266486 (‘NU-AGE: New dietary strategies addressing the specific needs of the elderly population for healthy aging in Europe’); the European Union’s Seventh Framework Program under grant agreement n° 259679 (‘IDEAL: Integrated research on Developmental determinants of Aging and Longevity’); MIUR Prin 2009 to CF; Roberto and Cornelia Pallotti Legacy for cancer research to SS and MC.

References


replicationsense cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements. Aging Cell 12, 247–256.


ARTICLE IN PRESS


Sacco, R., Ray, Rando, Post, 14 G

http://dx.doi.org/10.1016/j.mad.2013.12.006


Weilner, Weisenberger, Wahlgren, Vasto, Weiss, Wilson, Vaquero, G.

http://dx.doi.org/10.1016/j.mad.2013.12.006