

Epigenetics of Aging

Marta I. Sierra, Agustín F. Fernández and Mario F. Fraga*

Cancer Epigenetics Laboratory, Institute of Oncology of Asturias (IUOPA), HUCA, Universidad de Oviedo and Nanomaterials and Nanotechnology Research Center (CINN-CSIC)-Universidad de Oviedo (UO) -Principado de Asturias, Spain

Abstract: The best-known phenomenon exemplifying epigenetic drift (the alteration of epigenetic patterns during aging) is the gradual decrease of global DNA methylation. Aging cells, different tissue types, as well as a variety of human diseases possess their own distinct DNA methylation profiles, although the functional impact of these is not always clear. DNA methylation appears to be a dynamic tool of transcriptional regulation, with an extra layer of complexity due to the recent discovery of the conversion of 5-methylcytosine into 5-hydroxymethylcytosine. This age-related DNA demethylation is associated with changes in histone modification patterns and, furthermore, we now know that ncRNAs have evolved in eukaryotes as epigenetic regulators of gene expression. In this review, we will discuss current knowledge on how all these epigenetic phenomena are implicated in human aging, and their links with external, internal and stochastic factors which can affect human age-related diseases onset.

Keywords: Aging, Age-related diseases, DNA methylation, Epigenetics, External factors, Histone modifications, Non-coding RNAs.

EPIGENETICS OF AGING

According to the dictionary, aging means “to grow old”; but more in keeping with the aim of this chapter, we will center our discussion on aging as “the time-dependent functional decline that affects most living organisms” [1]. Of the nine hallmarks of aging that these authors propose, this review will deal with that of epigenetic alterations, the other eight being: genomic instability, telomere attrition, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Taken together, these events enable the identification and categorization of the cellular and molecular events leading to an aging phenotype.

First of all, and continuing with definitions, we will use epigenetic regulation to refer to the biological mechanisms in which DNA, RNA, and proteins are chemically or structurally modified, without changing their primary sequence. These epigenetic modifications play critical roles in the regulation of numerous cellular processes, including gene expression, DNA replication, and recombination. Epigenetic regulatory mechanisms include, among others, DNA methylation and hydroxymethylation, histone modification, chromatin remodeling, RNA methylation, and regulation by small and long non-coding RNAs. While epigenetic modifications can be very stable, and passed on to multiple generations in some cases, they can also change dynamically in response to specific cellular conditions or environmental stimuli. When

epigenetic mechanisms are misregulated, the result can be detrimental to health and they are therefore, emerging as important diagnostic and/or prognostic biomarkers in many fields of medicine.

BEST KNOWN EPIGENETIC MECHANISMS

Epigenetically regulated gene expression is a consequence of small covalent chemical modifications, which mark the genome and play a role in turning genes on or off. DNA methylation is one such mark. In this process, methyl groups attach to the backbone of the DNA molecule at cytosine rings found at CpG dinucleotides, a process catalyzed by DNA methyltransferases (DNMT3a/DNMT3b and DNMT1) [2]; in contrast demethylation can occur either passively, or by active mechanisms implicating ten-eleven translocator (TET) proteins and thymidine glycosidases [3]. These methyl groups typically turn genes off by affecting DNA accessibility.

Another type of epigenetic mark, collectively known as histone modifications, also affects accessibility of DNA, albeit indirectly. There are a variety of such chemical marks that modify the amino terminal tails of histones (e.g. acetylation, methylation, phosphorylation), changing how tightly or loosely DNA is packaged. In general, when the wrapping is tight, a gene is less accessible to the cellular transcription machinery, and consequently less expressed. When the wrapping is lost the gene generally becomes accessible. For example, histone deacetylation results in transcriptional repression, while histone acetylation, which involves the covalent addition of acetyl groups to the lysine moieties in the amino terminal histone tails, results in an increase in gene expression [4]. Other important epigenetic regulators of gene expression are non-coding RNAs (ncRNAs, transcripts that

*Address correspondence to this author at the Cancer Epigenetics Laboratory, Institute of Oncology of Asturias (IUOPA), HUCA, Universidad de Oviedo and Nanomaterials and Nanotechnology Research Center (CINN-CSIC)-Universidad de Oviedo (UO) -Principado de Asturias, Spain; Tel: +34 985733644 (Ext. 218); E-mail: mffraga@cinn.es

are not translated into protein), which can range in size from a few nucleotides to several kilobases. These ncRNAs can mediate both transcriptional and post-transcriptional gene silencing as well as activation [5].

EPIGENETIC CHANGES DURING LIFE TIME AND AGING

At specific time points, gene and mRNA expression requirements of cells and tissues have to be fulfilled by processes functioning in a dynamic way, and allowing for characteristic needs, such as epigenetic mechanisms. As mentioned earlier, epigenetic factors alter gene expression patterns without changing the underlying DNA/RNA or protein sequences. The association and distribution of these epigenetic factors along the genome determines specific gene expression programs that define the functional state of a cell at a given time point and in a specific environment. Consequently, their maintenance during mitosis is essential for the functional stability of tissues over time. However, epigenetic factors can be stochastically gained or lost, increasing the functional heterogeneity of cells within a given tissue, which can drive the appearance of some phenotypes, such as those typically observed during aging.

Alteration of epigenetic patterns during aging is a phenomenon known as epigenetic drift [6], one of the best known being a gradual decrease in global DNA methylation with advancing age, described almost 30 years ago [7]. Changes in DNA methylation with aging have been reported in human tissues, as well as in a number of other species [8]. It has also been confirmed that aging cells, different tissue types, as well as a variety of human diseases possess their own distinct DNA methylation profiles [9], although the functional impact of these methylation patterns is not always clear. It seems that DNA methylation may not be restricted to gene silencing (as happens when it occurs at promoter sequences), since many actively transcribed genes show high levels of cytosine methylation in their gene bodies. Thus, DNA methylation would appear to be a dynamic tool of transcriptional regulation, with an extra layer of complexity due to the recent discovery of the conversion of 5-methylcytosine into 5-hydroxymethylcytosine, catalyzed by the tet (ten-eleven-translocation) dioxygenases family enzymes (TET1-3) [10-12].

Currently, we know that DNA methylation changes are rapidly acquired during childhood [13] and, often they tend to correlate with age in adults [14]. This correlation was recently established with Illumina Bead Chip array data, measuring more than 480000 CpGs islands, which yielded predictions of age with a mean deviation from chronological age of less than five years [14]. Subsequently, an epigenetic age-predictor was described for blood samples, based on pyrosequencing of only three CpGs [15]. However, it must be noted that, although these methods can be useful to identify relevant factors affecting healthy aging, their utility as predictors for overall survival or disease-free survival is not yet established.

Recently, a catalogue of 794 age-modified CpG sites that reflect the changes in DNA methylation levels taking place in blood leukocytes of human infants (3 to 60 months) has been published [16]. The authors describe that the genomic

location of these modified CpG sites differed depending on whether they were methylated or demethylated with age; and they postulate that these methylation changes could correspond to a program with potential functional relevance in leukocyte biology during the period of life studied, and not simply be due to a stochastic DNA methylation drift.

In fact, these results corroborate the previously proposed hypothesis that systemic DNA methylation changes are restricted to specific loci, and that cell type plays an important role in the regulation these methylation changes over time [17]. In this sense, another set of epigenetic mechanisms also emerge as relevant: the histone modifications. Age-related DNA demethylation is associated with changes in histone modification patterns [18]. For example, global expressions of histone modifications, acetylation of histone 3 lysine 9 (H3K9Ac) [19] and trimethylation of histone 3 lysine 27 (H3K27me3) [20] have been shown to decrease with age in normal cells. We now know, however, that the genes hypermethylated in blood during aging are associated with the presence of bivalent chromatin domains in embryonic stem cells [9, 21, 22] and with repressive histone marks (H3K27me3/H3K9me3) in differentiated cells [21]. Furthermore, the same repressive histone marks which are present in differentiated cells are also present in sequences of mesenchymal stem cells which are hypermethylated during aging, implying that, independent of morphogenic potential and/or cell type, these repressive histone marks are associated with DNA methylation gain during aging [23]. Moreover, our lab has also described that DNA sequences hypomethylated in mesenchymal stem cells and differentiated cells during aging are strongly enriched in the active chromatin mark H3K4me1; suggesting that this histone modification is a cell type-independent chromatin signature of DNA hypomethylation during aging [23].

Expression of ncRNAs has also been shown to change during life-time. ncRNAs have evolved in eukaryotes as epigenetic regulators of gene expression [24]. The most abundant regulatory ncRNAs are the 20-24nt small microRNAs (miRNAs), and the long non-coding RNAs (lncRNAs, <200nt). Each class of ncRNAs operates through distinct mechanisms, but their pathways for gene expression regulation seem to be interrelated [25]. miRNAs mostly function as endogenous repressors of target genes; and, in this way, they regulate multiple cellular processes, including proliferation, apoptosis, senescence, differentiation and development.

The global miRNA profiles associated with human aging have been examined in peripheral blood mononuclear cells. The results showed that a cohort of 144 miRNAs was suppressed in elderly individuals, while 21 miRNAs increased with age [26].

In general, the role of miRNAs in aging remains poorly understood although the aforementioned study, and others, provide evidence that miRNAs can affect pathways involved in aging, and cellular senescence. Since all these aspects have recently been reviewed [27], we refer the reader to this work for further clarification and detail of this issue.

Discovered during the 1970s, the most abundant internal mRNA/lncRNA modification is N6-methyl adenosine (m⁶A), present, on average, in more than 3 sites per mRNA mole-

cule (reviewed in [28]). Despite it being long known about, interest in mRNA/lncRNA modification was revived in 2011 upon the discovery that mA modification is the cellular substrate for the human enzyme FTO (fat mass and obesity associated [29]). Like TETs, FTO is an α -ketoglutarate dependent dioxygenase which catalyzes the demethylation of mA. This fact has prompted the idea that RNA de-modifications could act, in conjunction with the other epigenetic mechanisms already mentioned, as markers and controllers of aging.

EFFECTS OF EXTERNAL FACTORS ON EPIGENETIC CHANGES OVER TIME

There is compelling evidence demonstrating that the epigenome responds in a dynamic way to changes in the environment; controlling normal development, homeostasis, aging, and mediating responses to environmental stimuli.

In the lifestyle field, gene-diet interactions often rely on epigenetic mechanisms, which gave rise to the idea of nutrition having a role as an “epigenetic medicine” [30]. Continuing on this theme, a possible dietary manipulation of miRNA families has been proposed [31] while by turning the idea around, the proposition that epigenetics could be the key to personalized nutrition has been launched [32].

Furthermore, several stages of human development and food habits have been studied for associations between diet and epigenetic alterations, starting with maternal nutrition [33], and continuing with diets: high-fat [31, 34] and alcohol consumption [35]. Lately, an emerging body of data point to the role of other dietary factors (eg, polyphenols, catechins, bioflavonoids, etc) in the DNA methylation process [36].

The effects of some environmental chemicals on DNA methylation of human adults have been the subject of a recent review [37] looking at epidemiologic studies evaluating the association between DNA methylation levels and cadmium, lead, mercury, nickel, persistent organic pollutants, bisphenol A, polycyclic aromatic hydrocarbons, and phthalates. The conclusions, however, highlighted drawbacks in the published works that limit the possibility of drawing definitive conclusions [37]. More convincing is the association between heavy metals and epigenetic changes (reviewed in [38]); benzene [39]; traffic particles [40] and asbestos [35]. Other human habits like tobacco smoking [41], sun exposure [42], physical exercise [43] and use of oral contraceptives [44] have been found to be associated with epigenetic alterations.

EPIGENETICS IN AGE RELATED DISEASES

Aging is characterized by increasing morbidity and functional decline which eventually results in the death of an organism. In humans, aging is the largest risk factor for numerous diseases, including: cancer, Alzheimer’s disease, metabolic disorders (particularly diabetes), atherosclerosis, muscle and bone mass loss, hearing and visual decline, etc. Even though the links between epigenetics and these diseases are not the subject of this review, and they are discussed in depth elsewhere, we would like to briefly state the common/shared trends between aging and several of these pathologic conditions.

Cancer

Probably, the most studied of these diseases from the epigenetic perspective is cancer (reviewed in [45]). Briefly, like in aging, global DNA hypomethylation is a characteristic of cancer, whose main tumorigenic effect could be considered to be the creation of genomic instability since chromosomal breaks, translocations, and allelic losses are all facilitated by DNA hypomethylation [46]. Moreover, the hypomethylation-cancer association may reside in: the up-regulation of certain genes (and miRNAs), the disturbance of imprinting patterns, and/or the transcriptional activation of parasitic sequences resident in the patient’s DNA (transposon elements, virus, etc.). The other DNA methylation signature of cancer is the aberrant hypermethylation of tumor suppressor genes, a phenomenon also observed in aging [47].

However, DNA methylation is involved in tumorigenesis in several other ways too, such as: somatic mutations in methyltransferases, as is the case in acute myeloid leukemia [48]; mutations due to the spontaneous deamination of 5-methylcytosine, yielding thymine, that can act as an endogenous mutagen [49]; and aberrant hypermethylation of normally unmethylated promoter CpG islands, yielding silencing of, for instance, tumor suppressor genes [45].

Recently, the study of the relation between DNA methylation and age, from a large number of samples of normal and cancer tissues (from several previous investigations), showed that the characteristics of the genomic regions involved in the normal age signature were quite different from those of the cancer signature [50]. However, with age, the DNA methylation levels of the normal signature approached the corresponding cancer levels, and the particular increases or decreases in age-associated methylation of the normal signature were aberrantly accelerated in cancer samples [50].

Of note is a recent report demonstrating that the prognostic impact of epigenetic markers in colorectal cancer patients, reverses with advancing age; giving rise to the hypothesis that colorectal cancer biology is epigenetically different in young and elderly patients [51].

Bone Density and Sarcopenia

Aging is accompanied by a reduction in muscle mass and function, commonly referred to as sarcopenia, leading to decreased mobility. Different biochemical pathways have been associated with it, and recently, the development of this age-related disorder has been shown to be linked to DNA methylation in aging postmitotic skeletal muscle [52].

Furthermore, characteristic age-associated bone density loss has also recently been related to epigenetic mechanisms. In this case, miRNAs seem to impact the osteogenic lineage commitment needed for bone development and homeostasis. This regulation of the skeleton by ncRNAs, could be translated into therapeutic targets for this common age-related disorder [53].

Alzheimer’s Disease

Alzheimer’s disease (AD) is an age-related neurodegenerative disorder, and probably the most common form of dementia in elderly humans. Clinically, it is characterized by

progressive memory loss and cognitive impairment. Evidence for the involvement of amyloid β metabolism in AD has emerged from linkage analyses, and from recent genome-wide association studies. However, the moderate concordance of disease development among twins suggests that other factors, potentially epigenomic factors, are related to AD. Available data point to AD brains showing slight DNA hypomethylation compared with normal brains, down-regulation of H3 K18/K23 acetylation marks, and highly variable results concerning miRNAs, with some of them upregulated and others downregulated. We will not however discuss this topic further here since current knowledge has recently been reviewed [54].

Cardiovascular Diseases, Including Atherosclerosis

In recent years, the impact of epigenetic mechanisms in cardiovascular patho-physiology has become increasingly recognized as a major factor in the interface between genotype and environment [55]. Genetic predisposition is a significant factor in atherosclerosis, but genome-wide association studies suggest that only $\approx 10\%$ of cases of cardiovascular disease have a heritable component. Studies with matched, atherosclerotic and normal, aortic samples from donors showed DNA hypermethylation in many loci from the atherosclerotic portion, compared with the healthy counterpart [56]. These authors identified the genome-wide DNA methylation changes and the locus-specific CpG alterations taking place during the onset and progression of human atherosclerotic lesions.

More recently, the same research group reported the CpG loci in the vascular lesions genome undergoing a DNA methylation drift with atheroma lesion progression. Surprisingly, the majority of these atherosclerosis progression-specific DNA methylation profiles were drifting towards hypermethylation with lesion progression [57], the surprising part coming from the fact that this increase in DNA methylation represents a singular epigenomic profile compared with other blood-related diseases, tumoral transformations, and/or aging.

Metabolism

A genome-wide age-associated DNA methylation study performed on peripheral blood of individuals at high risks of metabolic syndrome has recently been published [58]. The individuals, aged from 6 to 85, were from seven families, and 73% of the adults and 32% of the children were overweight or obese. Age-associated DNA methylation sites were concentrated around genes working in pathways such as the hedgehog signaling and the MODY (maturity-onset diabetes of the young) pathways. The researchers also observed that several genes known to be related to metabolic syndrome showed differential epigenetic responses to age in individuals with and without this condition [58].

These findings suggested the existence of connections between epigenomic profiles and the risk and prevalence of obesity, metabolic syndrome, and diabetes. This idea has been reinforced by the, recently communicated, impact of age, BMI and HbA1c on the epigenetic variation (DNA methylation) of candidate genes for obesity, type 2 diabetes, and cancer in human adipose tissue [59]. Importantly, these

authors also demonstrate that, epigenetic biomarkers in blood can mirror the age-related epigenetic signatures in adipose tissue, the target tissues for metabolic diseases.

CONCLUDING REMARKS

Ultimately, the epigenome compiles information on genes being transcribed, on those poised for transcription, and on those not accessible to the transcriptional machinery at any given time.

Knowledge of the influence of internal and external factors on the epigenome, could lead to targeted epigenomic modifications able to reverse the effect of risk factors in disease onset. This could be especially useful for the noncommunicable diseases, associated with older age groups, such as cancers, cardiovascular diseases, chronic respiratory diseases, and/or diabetes, which show a global growing trend.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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