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RESEARCH ARTICLE

The epigenetic landscape of age-related diseases: the geroscience perspective

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Abstract In this review, we summarize current knowledge regarding the epigenetics of age-related diseases, focusing on those studies that have described DNA methylation landscape in cardio-vascular diseases, musculoskeletal function and frailty. We stress the importance of adopting the conceptual framework of "geroscience", which starts from the observation that advanced age is the major risk factor for several of these pathologies and aims at identifying the mechanistic links between aging and age-related diseases. DNA methylation undergoes a profound remodeling during aging, which includes global hypomethylation of the genome, hypermethylation at specific loci and an increase in inter-individual variation and in stochastic changes of DNA methylation values. These epigenetic modifications can be an important contributor to the development of age-related diseases, but our understanding on the complex relationship between the epigenetic signatures of aging and age-related disease is still poor. The most relevant results in this field come from the use of the so called "epigenetics clocks" in cohorts of subjects affected by age-related diseases. We report these studies in final section of this review.

Keywords Aging · Age-related diseases · Epigenetics · DNA methylation

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Aging is a multifactorial and complex process, characterized by a gradual decline in physiological functions, associated with decreased fitness and higher risk of major diseases. Recent conceptualizations have proposed a set of pillars, that is processes that promote aging in a highly interconnected way (Mahmoudi and Brunet 2012; López-Otín et al. 2013; Kennedy et al. 2014). Among these pillars, epigenetic mechanisms have emerged as playing a major role in aging and became an important subject of aging biology research.

DNA methylation, on which this review will focus on, is one of the most studied and best-characterized epigenetic modifications which substantially changes during aging. It consists in the covalent addition of a methyl group to the carbon-5 position of cytosine residues in CpG dinucleotides in genomic DNA (Jones 2012; Bergman and Cedar 2013). The bulk of the genome is markedly depleted in CpG sites, which on the contrary they are enriched in specific tracts of the genome, named CpG islands, often associated with gene promoters and regulatory regions. The methylation status of CpG islands and surrounding regions (CpG island shores) is an important factor in regulating chromatin structure and gene expression during development and differentiation (Deaton and Bird 2011). DNA methylation is strongly affected by environmental factors, including diet (Bacalini et al. 2014; Johnson and Belshaw 2014; Barrès and Zierath 2016), pollution (Lillycrop and Burdge 2014; Cao 2015) and stress (Gassen et al. 2016). Throughout lifespan, profound alterations in DNA methylation patterns occur (Weidner and Wagner 2014; Zampieri et al. 2015). Specifically, during aging, as reported by data obtained from several human tissues, three different types of changes occur. First, global DNA methylation level decreases with age. Global hypomethylation occurs principally in repetitive DNA sequences and is considered associated with increased activation of transposable elements (Alexeeff et al. 2013). In addition to global hypomethylation, aging results in differential methylation (mainly hypermethylation) of certain genomic loci, consistently observed within large human cohorts (Maegawa et al. 2010; Rakyan et al. 2010; Grönniger et al. 2010; Hernandez et al. 2011; Bocker et al. 2011; Bell et al. 2012; Heyn et al. 2012; Beerman et al. 2013; McClay et al. 2014; Reynolds et al. 2014; Bacalini et al. 2015). Finally, with advanced age, it is observed an increase in inter-individual divergence between patterns of DNA methylation, referred as epigenetic drift (Fraga et al. 2005; Bjornsson et al. 2008; Talens et al. 2012; Yuan et al. 2015; Slieker et al. 2016), and in stochastic epigenetic mutations (Gentilini et al. 2015).

A large body of evidence indicates that age-dependent DNA methylation remodelling is largely recapitulated in cancer (Baylin and Jones 2011; Klutstein et al. 2016) and, according to several authors, epigenetic changes that occur during physiological aging can increase the risk of tumor onset and progression (Lin and Wagner 2015; Klutstein et al. 2016). Paradoxically, while the intimate relationship between the epigenetic changes that occur in aging and those that occur in cancer has been largely discussed, this conceptual framework has been largely neglected in the study of other age-related diseases. It is well established that aging increases susceptibility to many diseases and it is considered as the largest risk factor for some of the most prevalent illness in Western countries. Nevertheless, as we will show in this review, most of the studies that have investigated the epigenetic landscape of age-related diseases accurately described the loci in which DNA methylation changes occur, but did not discuss their relationship with the patterns characteristic of physiological aging. This new approach in the study of age-related diseases is part of the "geroscience" conceptual framework (Kennedy et al. 2014), which highlights the large interconnection between aging and age-related diseases and aims to systematically identify the mechanistic cellular and molecular connections between the two phenomena. In the epigenetics field, the "aging perspective" in the study of age-related diseases has been appreciated only recently thanks to availability of the so-called "epigenetic clocks" (Bocklandt et al. 2011; Horvath 2013; Hannum et al. 2013; Weidner

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et al. 2014; Snir et al. 2016), that is mathematical models calibrated on the DNA methylation changes that occur during physiological aging. As we will show, samples from subjects suffering of age-related diseases tend to be epigenetically older than healthy individuals according to these clocks, suggesting that age-related diseases are characterized by the acceleration of the DNA methylation changes that normally occur during physiological aging.

In the following sections, we will discuss current knowledge on the DNA methylation patterns of age-related diseases and their relationship with age-specific patterns, focusing on cardio-vascular diseases (CVD), musculoskeletal function and frailty.

Epigenetics and age-related cardiovascular diseases

Atherosclerosis, CVD and their complications are a major cause of mortality and morbidity. Their incidence and prevalence dramatically increase with age, and aging is considered as a major intrinsic cardiovascular risk factor. Impact of epigenetic mechanisms in cardiovascular physiopathology represents one of the hottest research topics of the last years. Numerous risk factors of CVD have been identified since many years and role of DNA methylation, by its position at the interface between genes and environment, has been deeply investigated. Thus, regarding dyslipidaemia, epigenome-wide association studies (EWAS) have been carried out, identifying genes with different methylation profiles among affected individuals and controls. Carnitine palmitoyltransferase-1A (*CPT1A*) gene has emerged from the comparison of CD4 + T cells isolated from a large cohort of individuals (991 subjects from the GOLDN study): methylation at some specific CpG sites, located in intron 1 of *CPT1A,* have been found to be associated with very-low density lipoprotein (VLDL), low-density lipoprotein (LDL) and triglycerides (TG) blood parameters (Frazier-Wood et al. 2014; Irvin et al. 2014). Association between DNA methylation at the identified CpG sites and TG profile was replicated in the Framingham Heart Study (Irvin et al. 2014). *CPT1A* plays an important role in maintaining a healthy lipid profile, by its involvement in mitochondrial β-oxidation of long chain fatty acids. An increase in its expression may be associated with a favourable lipid profile, but it is still not clear whether an increase in DNA methylation determines a higher or lower *CPT1A* expression level. This gene has also emerged from another study, where the association between blood lipid levels and whole blood DNA methylation has been evaluated on 1776 subjects from another cohort (Pfeiffer et al. 2015). In this study, *CPT1A* gene product has been found to interact with 2 other proteins, encoded by ABC-transporter G1 (*ABCG1*) and sterol regulatory element-binding transcription factor 1 (*MIR33B/ SREBF1*) genes. *SREBF1* enhances lipogenesis by activating the synthesis of fatty acids and cholesterol, and its expression is simultaneous to that of *MIR33B* (Marquart et al. 2010). Activation of this pathway can determine impairment of both fatty acids metabolism and cholesterol homeostasis.

Some other genes have been found differentially methylated between individuals with dyslipidaemia and controls. For example, in a cohort of patients with familial hypercholesterolaemia, hypermethylation of the promoter region of ATP-binding cassette A1 (*ABCA1*), which encodes a protein involved in cholesterol transfer from blood to high-density lipoprotein (HDL) particles, was found to be associated with lower levels of HDL-cholesterol and with prior history of coronary artery disease (CAD) (Guay et al. 2012). Furthermore, methylation of apolipoprotein E (*APOE*) was recently found to be associated with total cholesterol plasma levels (Ma et al. 2015).

Unbalanced lipid accumulation establishes a vicious circle in which chronic inflammation of blood vessels walls gives rise to immune activation that, in turn, foster inflammation and immune cells recruitment, leading to the luminal narrowing characteristic of atherosclerosis (Weber and Noels 2011). As regards atherosclerosis, an EWAS on matched healthy and atherosclerotic human aortic samples was carried out and a specific epigenomic profile has been identified (Zaina et al. 2014). This atherosclerotic signature includes genes involved in crucial processes such as, among others, activity of endothelial and vascular smooth muscle cells (SMCs), monocytes migration, angiogenesis, blood coagulation and atherosclerotic plaque instability and size. Along with previous results, in atherosclerotic lesions, both a global hypermethylation of Alu sequences (Kim et al. 2010) and a reduction of the expression level of enzymes that mediate active DNA demethylation (Liu et al. 2013) were detected (Zaina et al. 2014).

Recently, the same research group identified specific CpG loci that undergo DNA methylation drift towards hypermethylation in parallel to the atherosclerosis lesions progression (del Pilar Valencia-Morales et al. 2015), reporting this way a possible promising epigenomic marker of CVD disease.

Of particular interest in atherosclerosis is the study of the methylation status of estrogen receptor-genes (ER) whose protective functions have been described for cardiovascular system (Mendelsohn and Karas 1999). Methylation of ESR-α gene was found to be increased in vascular specimens displaying atherosclerotic lesions (Post et al. 1999) and it was also observed a same pattern of DNA methylation changes in SMCs displaying a proliferative phenotype compared to cells originated from non-atherosclerotic aortas (Ying et al. 2000). Migration and proliferation of SMCs are involved in vascular injury and importance of another gene, named collagen type XV alpha 1 (COL15A1), has emerged from in vitro studies on proliferating SMCs. COL15A1 is an extracellular matrix protein that can modulate the SMCs phenotype, acting on their ability to proliferate and migrate. Interestingly, methylation level of COL15A1 gene changes according to the number of replications of SMCs (Connelly et al. 2013). A passage-dependent DNA methylation increase was also reported for ER-β gene, that was found to be hypermethylated when plaque and non-plaque regions were compared (Kim et al. 2007). The observed hypermethylation trend with increasing replicative passage number is similar to that that may occur physiologically with aging.

Regarding CAD, one of the most common consequence of atherosclerosis, hypermethylation of specific DMRs was observed in an EWAS performed in patients with ischemic cardiopathy (Sharma et al. 2014). Some of these DMRs are located in genes important for cardiovascular system, including C1QL4, CCDC47, transforming growth factor, beta receptor III (TGFBR3), ADA, HAND2 and IKIP. In particular, TGFBR3 plays a crucial role in maintaining cardiovascular health, as it inhibits TGF-β1 signalling, acting this way as an anti-fibrosis factor (Liang et al. 2012). Interestingly, a treatment with simvastatin up-regulates TGFBR3 expression, limiting this way cardiac fibrosis, by suppressing ERK1/2 and JNK-dependent fibroblast activity and collagen production (Sun et al. 2015). Otherwise, ADA has been found to be involved in the control of inflammation hypertension-

dependent mediated by physical activity (Cardoso et al. 2015). Genetic variants within this gene are associated to the pathogenesis of chronic heart failure (He et al. 2014) and CAD (Safranow et al. 2007; Saccucci et al. 2014). Finally, HAND2 is a cardiomyocyte marker, involved in cardiogenesis and coronary vasculogenesis (VanDusen et al. 2014) as well as cardiovascular system development (Dirkx et al. 2013).

There is a growing body of evidence that environment and lifestyle, i.e. diet, smoking, alcohol consumption or physical activity can modulate cardiovascular risk by changing DNA methylation profiles. As an example, it has been recently demonstrated that DNA methylation can mediate the health benefits gained from regular physical exercise training (Denham et al. 2015): a group of 12 healthy young men underwent a sprint interval training three times a week and genome-wide leukocyte DNA methylation was measured before and after 4 weeks. A general demethylation was observed in CpG islands and in gene promoters, suggesting a corresponding change in the genomic expression profile. Particularly interesting is the demethylation of epidermal growth factor (EGF) gene, associated with a decreased mRNA expression. This can result in an anti-atherogenic effect, since it can limit the activity of EGF receptor that mediates inflammation and vessel damage (Makki et al. 2013).

Epigenetics and musculoskeletal function during aging

Sarcopenia

Musculoskeletal system is greatly affected during aging, leading to decreased mobility. Adult skeletal muscle (SM) is characterized by a progressive loss of mass, strength and functionality. This condition, caused by the replacement of SM with adipose and fibrotic tissue and by the decrease in the regenerative capacity, is known as sarcopenia (Walston 2012). Progressive accumulation of non-contractile tissue results in functional impairment and physical disability in the elderly. Different molecular mechanisms, including epigenetics, regulate maintenance of muscle mass and sarcopenia could result from a dysregulation of these processes.

Regenerative capability of SM tissue is effected by satellite cells, the muscle stem cells located in niche, between basal lamina and sarcolemma (Yin et al. 2013). In physiological conditions, SM is characterized by a low-grade proliferation and in the absence of stimuli from the microenvironment, satellite cells are quiescent. However, they are able to escape from this state and rapidly proliferate and differentiate in response to injury or other stressors. Quiescence has long been viewed as a dormant state, but recent insights indicate an actively and strictly control of quiescence to maintain a poised state that permits rapid activation (Cheung and Rando 2013). Regenerative process in adult muscle, initiated by injury, stress or pathological signals, is activated through the paired box protein 7 (Pax7), a transcription factor that engage the myogenic program for new-myofibers generation (Kassar-Duchossoy et al. 2005; Relaix et al. 2006). Age-related changes of somatic stem cells cause an unbalanced between quiescent/proliferative cell number, causing a decline in their regenerative capability (Jung and Brack 2014). Epigenetic alterations in some crucial signaling pathways, like fibroblast growth factor receptor-1 (FGFR1), JAK/STAT or p38 MAPK, have been observed in satellite cells from aged mice (Segalés et al. 2016), contributing in compromised stem cell function. Thus, epigenetic changes seem to profoundly affect the function of stem cells and contribute to functional degradation (Pollina and Brunet 2011).

Of particular interest are the results of the first EWAS conducted on human SM, published by Zykovich et al. in 2014. Samples collected from healthy older adults were compared to younger ones (Zykovich et al. 2014). Authors identified several differential methylated CpG nucleotides between younger and older tissues and selected 500 CpG sites which were able to discriminate younger subjects from older ones. Aged muscle showed hypermethylation mainly in intragenic sequences (middle and 3′ end of genes), rather than in the promoter regions. Ontology analysis on intragenic regions methylation changes during aging revealed that the most enriched pathways were muscle functions and neuromuscular junctions.

Another environmental cue that could influence DNA methylation in SM cells is physical exercise, a factor that can maintain muscle mass and a better autonomy in older people. According to observational studies, mainly performed using blood samples, the effect appears moderate. However, some interventional studies examined specifically the impact of training on DNA methylation status of muscle tissue obtained by muscular biopsies. After acute training, it was observed a decreased in whole genome methylation (Barrès et al. 2012). Hypomethylation of promoters was associated with an increased expression of some genes involved in energy metabolism or mitochondrial function especially, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), pyruvate dehydrogenase kinase isoenzyme 4 (PDK4), or peroxisome proliferator-activated receptor γ (PPAR-γ), in a dose-dependent manner. These results suggest that DNA methylation changes can represent an active and adaptive response to exercise. More recently, Lindholm et al. performed an EWAS in a prospective study, in order to analyze methylation changes in human SM with training (Lindholm et al. 2014). To avoid any confounding factors, authors obtained an intra-individual control, training only one leg per subject. Through this innovative approach, they observed significantly modulation in DNA methylation after long-term endurance exercise training (3 months). Substantial increase of methylation level occurred mainly in regulatory and enhancer regions and was associated with a coordinated transcriptional response. Interestingly, modifications in methylation levels seemed to be a precise process, occurring in genes involved in structural changes of muscle tissue, inflammation and immunological pathways. It should be noted that those studies were mostly performed on young and healthy adults and that, to date, no interventional study examined effects of training on DNA methylation status of SM cells in elderly people.

Interestingly, changes in genome-wide DNA methylation patterns after exercise were also observed in SM from individuals who are first-degree relative of patients with type 2 diabetes (Nitert et al. 2012): authors noticed epigenetic differences in muscle from patients who are at risk for diabetes (relative to their family history), as compared to muscle from patients who are not at risk for this disease. Genes differentially methylated are involved in muscle biological pathways, such as insulin, MAPK or calcium signaling. After 6 months of exercise, like in the other studies cited, epigenetics changes were observed, with a reduction in the number of genes which were found to be differentially methylated between the two groups

of subjects (38 post-intervention versus 65 genes prior).

Osteoarthritis

Osteoarthritis (OA) is a comorbidity frequently observed in the elderly. This degradation of articular cartilage is associated with chronic disability, increased risk of fall and loss of independence.

During the last 3 years, results of EWAS conducted on OA cartilage samples, in different centres, were reported (Delgado-Calle et al. 2013; Fernández-Tajes et al. 2014; Jeffries et al. 2014): these studies identified large numbers of differentially methylated CpG sites in OA cartilages, compared to controls [healthy controls (Fernández-Tajes et al. 2014) or osteoporotic patients (Delgado-Calle et al. 2013)]. Genes that were found to be differentially methylated were previously identified as implicated in OA pathogenesis by their roles in skeletal/cartilage development and inflammatory responses. It was also reported that, within the same joint, there is a significant differential CpG methylation between matched eroded or intact cartilage (Jeffries et al. 2014), whereas subchondral bone seemed to share the same epigenetic phenotype as overlying eroded cartilage (Jeffries et al. 2016). Methylation levels of some CpG sites on OA cartilage were correlated with the histopathologic disease severity score, assessed by modified Mankin score (Jeffries et al. 2014). Recently, Zhang et al. reported changes in DNA methylation profile of OA chondrocytes according to disease progression, with changes preferentially observed at last stage of OA (Zhang et al. 2016).

Epigenetics and frailty

Frailty is a clinical geriatric syndrome associated with an increased vulnerability following stressors (Chen et al. 2014). Phenotypic definition of frailty includes different criteria, such as weakness, slowness, low level of physical activity, exhaustion and weight loss. Frail and pre-frail states are common among older adults. They are complex states, resulting from the combination of poor physiologic reserves and weakness. They are associated with an increased risk of falls, disability, hospitalization and mortality (Fried et al. 2001). Sarcopenia, described above, is associated

and contributes greatly to the frailty syndrome. These two conditions represent a crucial health problem for the elderly.

Impact of changes in DNA methylation occurring during aging on frailty has been poorly specifically investigated. A first study showed that frail individuals, aged between 65 and 89 years old, exhibited lower global DNA methylation levels, compared to subjects who were pre-frail or non-frail (Bellizzi et al. 2012). A 7-years follow-up study, dealing with 37 subjects, revealed that a worsening in the frailty status was associated with significant decrease in global DNA methylation level. In 2014, Collerton et al. investigated, in a cohort of 321 subjects aged 85 years, associations between frailty and alterations in DNA methylation, both at global and specific levels. 21.8% of the subjects were frail and 58.6% pre-frail. Subjects who presented lower levels of DNA methylation at promoter specific CpG sites had decreased odds of frailty. Unlike the previous study, genome-wide methylation was not correlated with the frailty status (Collerton et al. 2014).

Epigenetic clock and association to morbidity and mortality

As mentioned above, during aging, hypermethylation and hypomethylation events, involving specific CpG sites, occur and are consistent between individuals. Some sites are highly associated with age and, using data obtained by EWAS, mathematical models were developed to identify potential biomarkers of aging, referred as epigenetic clock. Based on age-related changes in DNA methylation from multiple CpG sites across the genome, it is possible to accurately predict the biological age of an individual (Bocklandt et al. 2011; Horvath 2013; Hannum et al. 2013; Weidner et al. 2014; Snir et al. 2016). Thus, positive deviation of biological age compared to chronological age is considered as an age acceleration. Epigenetic clock results are informative of biological age of individuals, and therefore possibly associated to their health status and to potential occurrence of age-related health outcomes. At least four studies demonstrated that epigenetic clock in blood can predict all-cause mortality (Marioni et al. 2015a; Perna et al. 2016; Christiansen et al. 2016; Chen et al. 2016), even after taking consideration of a variety of other potential

confounding variables, including adjustment for blood cell composition. Moreover, it is associated with specific morbidity, such as CVD risk (Perna et al. 2016). Recently, biological age of patients with stroke was determined and compared to controls of the same chronological age: patients with ischemic stroke were biologically older than healthy controls. This was particularly true for younger patients (Soriano-Tárraga et al. 2016). Regarding OA, it was recently shown that cartilages of patients with OA demonstrate a specific accelerated aging as compared to controls, without differences in epigenetic aging of bone nearby and without a significant accelerated epigenetic aging in blood cells of OA patients (Vidal-Bralo et al. 2016). DNA methylation age correlates also with cognitive and physical illness: in a study involving 1091 older individuals, Marioni et al. observed that higher age acceleration was associated with lower abilities, such as fluid cognitive ability, grip strength or lung function (Marioni et al. 2015b). Finally, DNA methylation age was also found to be associated with frailty, assessed by an index corresponding to the proportion of deficits present among 34 items (Breitling et al. 2016).

Conclusion

Epigenetic changes have been identified as key players in normal and pathological aging and accurate description of the epigenetic landscape of age-related diseases has been provided in the last years. Nevertheless, our understanding of how age-associated epigenetic changes contribute to the epigenetic dysregulation and, more in general, to the pathology of age-related diseases is still at the beginning. The new conceptual framework of the "geroscience" (Kennedy et al. 2014) has focused the need to systematically investigate the links between aging and age-related pathologies, in order to understand how age enables disease. Currently, the most relevant result in this field is the acceleration in DNA methylation age observed in age-related diseases using Horvath's and Hannum's epigenetic clocks. This observation points for an intimate connection between epigenetic dysregulation in physiological and pathological aging, but it is often difficult to dissect the single components of these algorithms in order to identify the most relevant genomic loci affected by aging and potentially associated with the diseases. Therefore, future studies should explicitly investigate the existence of an overlap between DNA methylation signatures in chronological age and in age-related diseases, as it has been demonstrated for cancer (Lin and Wagner 2015), and identify those genomic loci that undergo epigenetic changes during chronological aging and that are also clearly affected in age-related diseases. Finally, future EWAS studies on age-related diseases should take into account not only the relationship between the observed epigenetic signatures and those characteristic of aging, but also the complex interaction with the (internal and external) environment, that can shape the epigenetic identity of each individual in different ways during life course. Lessons come from the field of genetics of age-related diseases, where it has been theorized and demonstrated that the association of a genetic variant with a disease can vary during the life of an individual and according to the environmental conditions that he/she encountered, strictly depending from the birth cohort (Franceschi et al. 2000; Ukraintseva et al. 2016; Franceschi and Garagnani 2016; Kulminski et al. 2016). Moreover, the study of the epigenetics of age-related diseases will certainly benefit from the use of longitudinally followed cohorts, which allow to take into account the complex relationship between age and pathologies. In conclusion, we foresee that the implementation of a geroscience perspective in the study of the epigenetics of age-related diseases will shed light into the mechanisms of the pathology and will pave the way for new therapeutical approaches, based on pharmacological, nutritional or physical interventions, that counteracting aging will counteract also age-related diseases.

Complaince with ethical standards

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References

Alexeeff SE, Baccarelli AA, Halonen J et al (2013) Association between blood pressure and DNA methylation of retrotransposons and pro-inflammatory genes. Int J Epidemiol 42:270–280. doi:10.1093/ije/dys220

Bacalini MG, Friso S, Olivieri F et al (2014) Present and future of anti-ageing epigenetic diets. Mech Ageing Dev 136–137:101–115. doi:10.1016/j.mad.2013.12.006

Bacalini MG, Boattini A, Gentilini D et al (2015) A meta-analysis on age-associated changes in blood DNA methylation: results from an original analysis pipeline for Infinium 450 k data. Aging 7:97–109. doi:10.18632/aging.100718

Barrès R, Zierath JR (2016) The role of diet and exercise in the transgenerational epigenetic landscape of T2DM. Nat Rev Endocrinol 12:441–451. doi:10.1038/nrendo.2016.87

Barrès R, Yan J, Egan B et al (2012) Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab 15:405–411. doi:10.1016/j.cmet.2012.01.001

Baylin SB, Jones PA (2011) A decade of exploring the cancer epigenome—biological and translational implications. Nat Rev Cancer 11:726–734. doi:10.1038/nrc3130

Beerman I, Bock C, Garrison BS et al (2013) Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. Cell Stem Cell 12:413–425. doi:10.1016/j.stem.2013.01.017

Bell JT, Tsai P-C, Yang T-P et al (2012) Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. PLoS Genet 8:e1002629. doi:10.1371/journal.pgen.1002629

Bellizzi D, D'Aquila P, Montesanto A et al (2012) Global DNA methylation in old subjects is correlated with frailty. Age Dordr Neth 34:169–179. doi:10.1007/s11357-011-9216-6

Bergman Y, Cedar H (2013) DNA methylation dynamics in health and disease. Nat Struct Mol Biol 20:274–281. doi:10.1038/nsmb.2518

Bjornsson HT, Sigurdsson MI, Fallin MD et al (2008) Intra-individual change over time in DNA methylation with familial clustering. JAMA 299:2877–2883. doi:10.1001/jama.299.24.2877

Bocker MT, Hellwig I, Breiling A et al (2011) Genome-wide promoter DNA methylation dynamics of human hematopoietic progenitor cells during differentiation and aging. Blood 117:e182–189. doi:10.1182/blood-2011-01-331926

Bocklandt S, Lin W, Sehl ME et al (2011) Epigenetic predictor of age. PloS ONE 6:e14821. doi:10.1371/journal.pone.0014821

Breitling LP, Saum K-U, Perna L et al (2016) Frailty is associated with the epigenetic clock but not with telomere length in a German cohort. Clin Epigenet 8:21. doi:10.1186/s13148-016-0186-5

Cao Y (2015) Environmental pollution and DNA methylation: carcinogenesis, clinical significance, and practical applications. Front Med 9:261–274. doi:10.1007/s11684-015-0406-y

Cardoso AM, Abdalla FH, Bagatini MD, et al (2015) Swimming training prevents alterations in ecto-NTPDase and adenosine deaminase activities in lymphocytes from Nω-nitro-L-arginine methyl ester hydrochloride induced hypertension rats. J Hypertens 33:763–772; discussion 772. doi:10.1097/HJH.0000000000000468

Chen X, Mao G, Leng SX (2014) Frailty syndrome: an overview. Clin Interv Aging 9:433–441. doi:10.2147/CIA.S45300

Chen BH, Marioni RE, Colicino E et al (2016) DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging 8:1844–1865. doi:10.18632/aging.101020

Cheung TH, Rando TA (2013) Molecular regulation of stem cell quiescence. Nat Rev Mol Cell Biol 14:329–340. doi:10.1038/nrm3591

Christiansen L, Lenart A, Tan Q et al (2016) DNA methylation age is associated with mortality in a longitudinal Danish twin study. Aging Cell 15:149–154. doi:10.1111/acel.12421

Collerton J, Gautrey HE, van Otterdijk SD et al (2014) Acquisition of aberrant DNA methylation is associated with frailty in the very old: findings from the Newcastle 85+ study. Biogerontology 15:317–328. doi:10.1007/s10522-014-9500-9

Connelly JJ, Cherepanova OA, Doss JF et al (2013) Epigenetic regulation of COL15A1 in smooth muscle cell replicative aging and atherosclerosis. Hum Mol Genet 22:5107–5120. doi:10.1093/hmg/ddt365

Deaton AM, Bird A (2011) CpG islands and the regulation of transcription. Genes Dev 25:1010–1022. doi:10.1101/gad.2037511

del Pilar Valencia-Morales M, Zaina S, Heyn H et al (2015) The DNA methylation drift of the atherosclerotic aorta increases with lesion progression. BMC Med Genomics 8:7. doi:10.1186/s12920-015-0085-1

Delgado-Calle J, Fernández AF, Sainz J et al (2013) Genome-wide profiling of bone reveals differentially methylated regions in osteoporosis and osteoarthritis. Arthritis Rheum 65:197–205. doi:10.1002/art.37753

Denham J, O'Brien BJ, Marques FZ, Charchar FJ (2015) Changes in the leukocyte methylome and its effect on cardiovascular-related genes after exercise. J Appl Physiol 118:475–488. doi:10.1152/japplphysiol.00878.2014

Dirkx E, Gladka MM, Philippen LE et al (2013) Nfat and miR-25 cooperate to reactivate the transcription factor Hand2 in heart failure. Nat Cell Biol 15:1282–1293. doi:10.1038/ncb2866

Fernández-Tajes J, Soto-Hermida A, Vázquez-Mosquera ME et al (2014) Genome-wide DNA methylation analysis of articular chondrocytes reveals a cluster of osteoarthritic patients. Ann Rheum Dis 73:668–677. doi:10.1136/annrheumdis-2012-202783

Fraga MF, Ballestar E, Paz MF et al (2005) Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci USA 102:10604–10609. doi:10.1073/pnas.0500398102

Franceschi C, Garagnani P (2016) Suggestions from geroscience for the genetics of age-related diseases. PLoS Genet 12:e1006399. doi:10.1371/journal.pgen.1006399

Franceschi C, Valensin S, Bonafè M et al (2000) The network and the remodeling theories of aging: historical background and new perspectives. Exp Gerontol 35:879–896

Frazier-Wood AC, Aslibekyan S, Absher DM et al (2014) Methylation at CPT1A locus is associated with lipoprotein subfraction profiles. J Lipid Res 55:1324–1330. doi:10.1194/jlr.M048504

Fried LP, Tangen CM, Walston J et al (2001) Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 56:M146–156

Gassen NC, Chrousos GP, Binder EB, Zannas AS (2016) Life stress, glucocorticoid signaling, and the aging epigenome: implications for aging-related diseases. Neurosci Biobehav Rev. doi:10.1016/j.neubiorev.2016.06.003

Gentilini D, Garagnani P, Pisoni S et al (2015) Stochastic epigenetic mutations (DNA methylation) increase exponentially in human aging and correlate with X chromosome inactivation skewing in females. Aging 7:568–578. doi:10.18632/aging.100792

Grönniger E, Weber B, Heil O et al (2010) Aging and chronic sun exposure cause distinct epigenetic changes in human skin. PLoS Genet 6:e1000971. doi:10.1371/journal.pgen.1000971

Guay S-P, Brisson D, Munger J et al (2012) ABCA1 gene promoter DNA methylation is associated with HDL particle profile and coronary artery disease in familial hypercholesterolemia. Epigenetics 7:464–472. doi:10.4161/epi.19633

Hannum G, Guinney J, Zhao L et al (2013) Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell 49:359–367. doi:10.1016/j.molcel.2012.10.016

He H-R, Li Y-J, He G-H et al (2014) The adenosine deaminase gene polymorphism is associated with chronic heart failure risk in Chinese. Int J Mol Sci 15:15259–15271. doi:10.3390/ijms150915259

Hernandez DG, Nalls MA, Gibbs JR et al (2011) Distinct DNA methylation changes highly correlated with chronological age in the human brain. Hum Mol Genet 20:1164–1172. doi:10.1093/hmg/ddq561

Heyn H, Li N, Ferreira HJ et al (2012) Distinct DNA methylomes of newborns and centenarians. Proc Natl Acad Sci USA 109:10522–10527. doi:10.1073/pnas.1120658109

Horvath S (2013) DNA methylation age of human tissues and cell types. Genome Biol 14:R115. doi:10.1186/gb-2013-14-10-r115

Irvin MR, Zhi D, Joehanes R et al (2014) Epigenome-wide association study of fasting blood lipids in the genetics of lipid-lowering drugs and diet network study. Circulation 130:565–572. doi:10.1161/CIRCULATIONAHA.114.009158

Jeffries MA, Donica M, Baker LW et al (2014) Genome-wide DNA methylation study identifies significant epigenomic changes in osteoarthritic cartilage. Arthritis Rheumatol Hoboken NJ 66:2804–2815. doi:10.1002/art.38762

Jeffries MA, Donica M, Baker LW et al (2016) Genome-wide DNA methylation study identifies significant epigenomic changes in osteoarthritic subchondral bone and similarity to overlying cartilage. Arthritis Rheumatol Hoboken NJ 68:1403–1414. doi:10.1002/art.39555

Johnson IT, Belshaw NJ (2014) The effect of diet on the intestinal epigenome. Epigenomics 6:239–251. doi:10.2217/epi.14.8

Jones PA (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 13:484–492. doi:10.1038/nrg3230

Jung Y, Brack AS (2014) Cellular mechanisms of somatic stem cell aging. Curr Top Dev Biol 107:405–438. doi:10.1016/B978-0-12-416022-4.00014-7

Kassar-Duchossoy L, Giacone E, Gayraud-Morel B et al (2005) Pax3/Pax7 mark a novel population of primitive myogenic cells during development. Genes Dev 19:1426–1431. doi:10.1101/gad.345505

Kennedy BK, Berger SL, Brunet A et al (2014) Geroscience: linking aging to chronic disease. Cell 159:709–713. doi:10.1016/j.cell.2014.10.039

Kim J, Kim JY, Song KS et al (2007) Epigenetic changes in estrogen receptor beta gene in atherosclerotic cardiovascular tissues and in vitro vascular senescence. Biochim Biophys Acta 1772:72–80. doi:10.1016/j.bbadis.2006.10.004

Kim M, Long TI, Arakawa K et al (2010) DNA methylation as a biomarker for cardiovascular disease risk. PLoS ONE 5:e9692. doi:10.1371/journal.pone.0009692

Klutstein M, Nejman D, Greenfield R, Cedar H (2016) DNA methylation in cancer and aging. Cancer Res 76:3446–3450. doi:10.1158/0008-5472.CAN-15-3278

Kulminski AM, He L, Culminskaya I et al (2016) Pleiotropic associations of allelic variants in a 2q22 region with risks of major human diseases and mortality. PLoS Genet 12:e1006314. doi:10.1371/journal.pgen.1006314

Liang H, Zhang C, Ban T et al (2012) A novel reciprocal loop between microRNA-21 and TGFβRIII is involved in cardiac fibrosis. Int J Biochem Cell Biol 44:2152–2160. doi:10.1016/j.biocel.2012.08.019

Lillycrop KA, Burdge GC (2014) Environmental challenge, epigenetic plasticity and the induction of altered phenotypes in mammals. Epigenomics 6:623–636. doi:10.2217/epi.14.51

Lin Q, Wagner W (2015) Epigenetic aging signatures are coherently modified in cancer. PLoS Genet 11:e1005334. doi:10.1371/journal.pgen.1005334

Lindholm ME, Marabita F, Gomez-Cabrero D et al (2014) An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. Epigenetics 9:1557–1569. doi:10.4161/15592294.2014.982445

Liu R, Jin Y, Tang WH et al (2013) Ten-eleven translocation-2 (TET2) is a master regulator of smooth muscle cell plasticity. Circulation 128:2047–2057. doi:10.1161/CIRCULATIONAHA.113.002887

López-Otín C, Blasco MA, Partridge L et al (2013) The hallmarks of aging. Cell 153:1194–1217. doi:10.1016/j.cell.2013.05.039

Ma Y, Smith CE, Lai C-Q et al (2015) Genetic variants modify the effect of age on APOE methylation in the genetics of lipid lowering drugs and diet network study. Aging Cell 14:49–59. doi:10.1111/acel.12293

Maegawa S, Hinkal G, Kim HS et al (2010) Widespread and tissue specific age-related DNA methylation changes in

mice. Genome Res 20:332–340. doi:10.1101/gr.096826.109

Mahmoudi S, Brunet A (2012) Aging and reprogramming: a two-way street. Curr Opin Cell Biol 24:744–756. doi:10.1016/j.ceb.2012.10.004

Makki N, Thiel KW, Miller FJ (2013) The epidermal growth factor receptor and its ligands in cardiovascular disease. Int J Mol Sci 14:20597–20613. doi:10.3390/ijms141020597

Marioni RE, Shah S, McRae AF et al (2015a) DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol 16:25. doi:10.1186/s13059-015-0584-6

Marioni RE, Shah S, McRae AF et al (2015b) The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. Int J Epidemiol 44:1388–1396. doi:10.1093/ije/dyu277

Marquart TJ, Allen RM, Ory DS, Baldán A (2010) miR-33 links SREBP-2 induction to repression of sterol transporters. Proc Natl Acad Sci USA 107:12228–12232. doi:10.1073/pnas.1005191107

McClay JL, Aberg KA, Clark SL et al (2014) A methylome-wide study of aging using massively parallel sequencing of the methyl-CpG-enriched genomic fraction from blood in over 700 subjects. Hum Mol Genet 23:1175–1185. doi:10.1093/hmg/ddt511

Mendelsohn ME, Karas RH (1999) The protective effects of estrogen on the cardiovascular system. N Engl J Med 340:1801–1811. doi:10.1056/NEJM199906103402306

Nitert MD, Dayeh T, Volkov P et al (2012) Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. Diabetes 61:3322–3332. doi:10.2337/db11-1653

Perna L, Zhang Y, Mons U et al (2016) Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. Clin Epigenetics 8:64. doi:10.1186/s13148-016-0228-z

Pfeiffer L, Wahl S, Pilling LC et al (2015) DNA methylation of lipid-related genes affects blood lipid levels. Circ Cardiovasc Genet 8:334–342. doi:10.1161/CIRCGENETICS.114.000804

Pollina EA, Brunet A (2011) Epigenetic regulation of aging stem cells. Oncogene 30:3105–3126. doi:10.1038/onc.2011.45

Post WS, Goldschmidt-Clermont PJ, Wilhide CC et al (1999) Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. Cardiovasc Res 43:985–991

Rakyan VK, Down TA, Maslau S et al (2010) Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res 20:434–439. doi:10.1101/gr.103101.109

Relaix F, Montarras D, Zaffran S et al (2006) Pax3 and Pax7 have distinct and overlapping functions in adult muscle progenitor cells. J Cell Biol 172:91–102. doi:10.1083/jcb.200508044

Reynolds LM, Taylor JR, Ding J et al (2014) Age-related variations in the methylome associated with gene expression in human monocytes and T cells. Nat Commun 5:5366. doi:10.1038/ncomms6366

Saccucci P, Binczak-Kuleta A, Banci M et al (2014) Coronary artery disease. A study of three polymorphic sites of

adenosine deaminase gene. Acta Cardiol 69:39–44. doi:10.2143/AC.69.1.3011343

Safranow K, Rzeuski R, Binczak-Kuleta A et al (2007) ADA*2 allele of the adenosine deaminase gene may protect against coronary artery disease. Cardiology 108:275–281. doi:10.1159/000099096

Segalés J, Perdiguero E, Muñoz-Cánoves P (2016) Regulation of muscle stem cell functions: a focus on the p38 MAPK signaling pathway. Front Cell Dev Biol 4:91. doi:10.3389/fcell.2016.00091

Sharma P, Garg G, Kumar A et al (2014) Genome wide DNA methylation profiling for epigenetic alteration in coronary artery disease patients. Gene 541:31–40. doi:10.1016/j.gene.2014.02.034

Slieker RC, van Iterson M, Luijk R et al (2016) Age-related accrual of methylomic variability is linked to fundamental ageing mechanisms. Genome Biol 17:191. doi:10.1186/s13059-016-1053-6

Snir S, vonHoldt BM, Pellegrini M (2016) A statistical framework to identify deviation from time linearity in epigenetic aging. PLoS Comput Biol 12:e1005183. doi:10.1371/journal.pcbi.1005183

Soriano-Tárraga C, Giralt-Steinhauer E, Mola-Caminal M et al (2016) Ischemic stroke patients are biologically older than their chronological age. Aging 8:2655–2666. doi:10.18632/aging.101028

Sun F, Duan W, Zhang Y et al (2015) Simvastatin alleviates cardiac fibrosis induced by infarction via up-regulation of TGF-β receptor III expression. Br J Pharmacol 172:3779–3792. doi:10.1111/bph.13166

Talens RP, Christensen K, Putter H et al (2012) Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11:694–703. doi:10.1111/j.1474-9726.2012.00835.x

Ukraintseva S, Yashin A, Arbeev K et al (2016) Puzzling role of genetic risk factors in human longevity: "risk alleles" as pro-longevity variants. Biogerontology 17:109–127. doi:10.1007/s10522-015-9600-1

VanDusen NJ, Casanovas J, Vincentz JW et al (2014) Hand2 is an essential regulator for two Notch-dependent functions within the embryonic endocardium. Cell Rep 9:2071–2083. doi:10.1016/j.celrep.2014.11.021

Vidal-Bralo L, Lopez-Golan Y, Mera-Varela A et al (2016) Specific premature epigenetic aging of cartilage in osteoarthritis. Aging 8:2222–2231. doi:10.18632/aging.101053

Walston JD (2012) Sarcopenia in older adults. Curr Opin Rheumatol 24:623–627. doi:10.1097/BOR.0b013e328358d59b

Weber C, Noels H (2011) Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 17:1410–1422. doi:10.1038/nm.2538

Weidner CI, Wagner W (2014) The epigenetic tracks of aging. Biol Chem 395:1307–1314. doi:10.1515/hsz-2014-0180

Weidner CI, Lin Q, Koch CM et al (2014) Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol 15:R24. doi:10.1186/gb-2014-15-2-r24

Yin H, Price F, Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. Physiol Rev 93:23–67. doi:10.1152/physrev.00043.2011

Ying AK, Hassanain HH, Roos CM et al (2000) Methylation of the estrogen receptor-alpha gene promoter is selectively increased in proliferating human aortic smooth muscle cells. Cardiovasc Res 46:172–179

Yuan T, Jiao Y, de Jong S et al (2015) An integrative multi-scale analysis of the dynamic DNA methylation landscape in aging. PLoS Genet 11:e1004996. doi:10.1371/journal.pgen.1004996

Zaina S, Heyn H, Carmona FJ et al (2014) DNA methylation map of human atherosclerosis. Circ Cardiovasc Genet 7:692–700. doi:10.1161/CIRCGENETICS.113.000441

Zampieri M, Ciccarone F, Calabrese R et al (2015) Reconfiguration of DNA methylation in aging. Mech Ageing Dev 151:60–70. doi:10.1016/j.mad.2015.02.002

Zhang Y, Fukui N, Yahata M et al (2016) Genome-wide DNA methylation profile implicates potential cartilage regeneration at the late stage of knee osteoarthritis. Osteoarthritis Cartilage 24:835–843. doi:10.1016/j.joca.2015.12.013

Zykovich A, Hubbard A, Flynn JM et al (2014) Genome-wide DNA methylation changes with age in disease-free human skeletal muscle. Aging Cell 13:360–366. doi:10.1111/acel.12180