Aging and DNA Methylation

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Abstract: Human longevity is influenced by both inherited and environmental factors. Alterations in gene function that are related to inherited genetic mutations and polymorphisms can explain some features of aging and age-related diseases. However, in addition to inherited genetic factors, aging is influenced by the gradual accumulation of molecular alterations after birth. Epigenetic changes can influence gene function during aging without inherited and acquired DNA sequence alterations. In particular, promoter DNA methylation changes and associated gene silencing are epigenetic changes that are prominent in some cells and age-related diseases. Here, we review genetic approaches to understand aging, and discuss the potential role of epigenetic mechanisms in human aging and age-related diseases.

Keywords: Aging, genetics, epigenetics, DNA methylation.

1. INTRODUCTION

1.1. Genetics in Aging: Applications and Limitations

Physiologic aging and associated diseases affect longevity and quality of life. A long, healthy lifespan is clearly influenced by genetic background, but is also affected by the gradual accumulation of molecular genetic alterations in cellular metabolism [1-3]. Several molecular genetic approaches have been used to understand factors related to the aging process and age-related diseases, but these remain incompletely understood.

Molecular genetic pathways that regulate the aging process have been studied using yeast, nematodes, flies, mouse models and tissues of primates [4-6]. Insulin/insulin-like growth factor 1 (IGF1) signaling has been shown to be a conserved regulatory system for aging in these model organisms [7,8]. And, silent information regulator (Sir2) of yeast and worms and its mammalian homolog sirtuins (SIRTs) are involved in longevity by the regulation of oxidative stress, DNA damage, glucose homeostasis, and adipogenesis in yeast, worms, flies, or mammals [9-11]. Even though supporting data are needed in humans, insulin/IGF1 and SIRT-related pathways may be the evidences of the genetic regulation of the aging process [12].

Genetic inheritance of longevity is evidence of the influence of genetics on aging. The inheritance of longevity was first hypothesized to be an evolutionary adaptation of the aging process, e.g., evolutionary senescence [13] and contrary function of pleiotropic genes during early and late aging process [14]. Some lines of evidence support this idea, such as findings of a trade-off between longevity and reproduction [15,16]. Longevity and the occurrence of age-related diseases tend to be inherited in some families [17] and show a high concordance rate in twins [18,19]. Additionally, aging itself increases the risk ratio of several diseases, e.g., diabetes mellitus, coronary heart disease, Alzheimer's disease and Parkinson's disease [20,21]. In some familial degenerative disorders, genetic alterations have been detected by statistical methods applied to biological and genetic data, e.g., *Notch3* for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) [22], mitochondrial DNA alterations for mitochondrial diseases [23], and *WRN* for Werner syndrome [24]. However, even with increased evidence for a genetic influence in the aging process, it is not easy to verify specific genetic abnormalities in many inherited age-related diseases due to a shortage of genetic data and limitations of the applied statistical methods [20].

Elucidating the genetic basis of age-related diseases is further complicated by the often weak association between specific diseases and previously-reported disease-specific genes. Indeed, if multiple polymorphisms in one gene can cause a specific disease, it will be difficult to understand the relationship between the genetic abnormality and functional protein alterations in that disease. For example, in potassium-related periodic paralysis, an autosomal dominant type of progressive muscular degeneration, at least 20 different novel polymorphisms in the skeletal muscle sodium channel gene (*SCN4A*) have been detected [25]. While these polymorphisms may cause protein alterations, the functional significance of each polymorphism is not yet understood. Recently, in a study of potassium-related periodic paralysis, a polymorphism found to be associated with hyperkalemic periodic paralysis was also shown to be associated with paramyotonia congenita [26]. These findings show the limitations of the one-gene/one-disease or one-polymorphism/one-disease outlooks.

Despite these limitations, several polymorphisms have been reported to be associated with age-related diseases, e.g., apolipoprotein allele E4 [27] and allele 2 of α2-macroglobulin [28] in Alzheimer's disease, the angiotensin-converting enzyme gene in myocardial infarction [29] and ischemic stroke [30], the endothelial nitric oxide synthase gene in ischemic stroke [31], and the E2 or E4 apolipoprotein allele

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1872-3136/09 $55.00+.00 © 2009 Bentham Science Publishers Ltd.

in intracerebral hemorrhage [32]. However, it has been difficult to confirm some of these relationships in subsequent studies [33-37].

Recently, large-scale studies of multiple, unselected polymorphisms associated with age-related disease such as myocardial infarction and stroke have produced interesting findings, identifying disease-related polymorphisms in some genes [38,39]. Nevertheless, single nucleotide polymorphisms in specific candidate genes have limited use as disease-related risk factors, because polymorphisms in specific genes may be altered by polymorphisms in other genes, and it is difficult to verify the functional role of each polymorphism in the candidate genes for these diseases [40].

1.2. Gradual Accumulation of Mitotically-Inherited Genetic Abnormalities

The progressive accumulation of altered DNA and/or RNA changes has been observed in mitotically-active cells, which have no evidence of meiotically-inherited genetic defects. Telomere shortening in *in vitro* and *in vivo* senescence is one such change [41-44]. However, telomere shortening is usually observed in replicating cells *in vitro* rather than in cells directly relevant to the aging process or age-related diseases. For example, in one study, stable telomere lengths were detected in brain tissue even though telomere shortening was detected in *in vitro* fibroblast replicative senescence [44]. Because of the potential disparities between *in vivo* and *in vitro* senescence, we must be cautious when extrapolating *in vitro* data to the human aging process [45].

The age-dependent accumulation of acquired somatic and mitochondrial DNA mutations could also be a genetic alteration associated with the aging process [46]. Oxidative stress and environmental factors during aging have been proposed as likely causes of acquired DNA mutations in susceptible age-related genes [46,47]. The accumulation of age-dependent genetic alterations is tissue-specific, and has been shown to be independent of cell proliferation rate in some transgenic mice models suitable for mutation assay [48]. For cancer development, in fact, multiple somatic DNA mutations have been identified as an important mechanism of tumorigenesis [47,49]. Recently, the accumulation of somatic DNA mutations has also been proposed as one of the genetic mechanisms involved in atherosclerosis pathogenesis [50]. The previous studies suggested the possibility of a relationship between non-inherited genetic alterations and the aging process. However, phenotypic alterations resulting from accumulated genetic alterations remain unclear. To verify the relationship between the age-related increase in genetic alterations and environmental causes, further evidence is required.

1.3. Epigenetic Gene Silencing in Mitotically-Active Cells

The heritability of longevity accounts for less than 35% of the human life span [1]. As described above, mitotically-inherited genetic modifications do occur, but provide a limited explanation of the disease processes associated with aging [51] and transformed cells [52].

Moreover, although genomic sequences are identical in most differentiated zygotic cells [53], in a given individual, one can observe focal defects in aging tissues, and differences in aging between different tissues. It is possible, then, that some of these age-related changes may be due to epigenetics, rather than genetics. Epigenetic modifications could cause gene expression changes in the aging process without associated coding sequence changes in the affected genes [53]. These epigenetic alterations can modify gene function in mitotically/meiotically-inherited cells [54]. Thus, epigenetic modifications may provide a plausible mechanism for understanding age-related alterations in gene expression.

CpG island methylation has been proposed as one of the most powerful molecular mechanisms of epigenetic gene silencing [53,55,56]. In this review, we will discuss observations and hypotheses regarding epigenetic modifications in normal aging and age-related diseases in humans, with an emphasis on DNA methylation and its role in the aging process.

2. WHAT IS DNA METHYLATION?

DNA methylation refers to the addition of a methyl (CH_3) group to a DNA molecule. DNA methylation in human somatic cells preferentially involves the 5' cytosine in CpG dinucleotides [57,58]. Although the frequency of CpG dinucleotides is relatively low in the human genome [59], they are enriched in 0.5- to 4-kb regions with a high CG%, known as CpG islands. These are often associated with genes and are distributed in different locations in the genome, including in promoters, exons, 5' flanking regions and 3' terminal areas [60,61]. Among the variably located CpG islands, promoter CpG islands in 5’ flanking regions have been the main focus of many epigenetic experiments that aim to understand the role of promoter CpG island-methylation in epigenetic gene silencing [57].

3. WHERE DOES DNA METHYLATION DEVELOP IN GENOMIC SEQUENCES?

3.1. Transcriptional Silencing by Promoter CpG Island Methylation

Almost half of the genes in the mammalian genome, including most housekeeping genes and half of all tissue-specific genes, have CpG islands in their proximal promoter area [59,61]. DNA-methylation in promoter CpG islands has been shown to be critical for epigenetic transcriptional gene silencing [57]. Furthermore, methylated CpG islands in the promoter region of transcriptionally-silenced genes switch the chromatin structure to a compact and closed conformation through the binding of methyl-cytosine-binding proteins, namely histone deacetylases and histone methylases [62]. Finally, the closed chromatin structure associated with methylated CpG islands can cause transcriptional repression of specific genes. Reactivation of silenced genes by demethylation and/or histone deacetylase inhibition supports the importance of promoter DNA methylation-protein interactions in epigenetic gene silencing [63]. Transcriptional gene repression associated with promoter CpG-island methylation has been proposed as an epigenetic mechanism that occurs in embryonic development [52], genomic imprinting [64], X chromosome inactivation [65] silencing of intragenomic parasites [66], carcinogenesis [66-68], the fragile X syndrome [69], as well as in the aging process [70] (described below).

3.2. Transcriptional Disturbances Due to CpG Island Methylation in Repetitive Sequences

Promoter DNA hypermethylation is an important epigenetic alteration, but promoter CpG islands account for less than one-fifth of the total number of CpG islands distributed in the human genome [60]. Although most CpG islands in the human genome are distributed in non-promoter sequences, the epigenetic characteristics and role of non-promoter CpG islands remain unclear.

Repetitive genomic sequences among the non-promoter CpG islands, including interspersed elements and tandem repeats, frequently have typical CpG islands and/or heterochromatic characteristics in their sequences. Studies to understand the epigenetic characteristics and functions of these repetitive elements have only recently emerged.

3.2.1. Interspersed Elements and CpG Island Methylation

Normal *de novo* methylation is established at a very early stage in embryo development, shortly after the erasure of methylation that happens from fertilization to the eight-cell stage [66,71,72]. After this developmentally programmed methylation is established, epigenetic information is thought to be relatively stable, with CpG island methylation and silencing limited to genomic imprinting [73] and X chromosome inactivation [74].

Interspersed repeats such as LINE-1 and *Alu* elements, also known as transposed intragenomic parasites, comprise 35-50% of the human genome [66]. These interspersed transposons are often rich in CpG sites and the changes in DNA methylation of these interspersed repeats parallels genome-wide changes in methylation during early embryonic development [75,76].

Genome-wide DNA methylation decreases in many tissue types during in *in vivo* aging and *in vitro* senescence [77-79]. Global demethylation has been suggested as a possible epigenetic alteration in cancer, resulting from aberrant promoter hypermethylation and DNMT overexpression [80,81]. Recently, hypomethylation of transposed elements such as *Alu* elements was also observed with increasing age [82,83], and demethylation of transposed sequences has been observed in cancer tissues and cell lines [84,85]. Demethylation of transposed elements such as LINEs and *Alu*s contributes significantly to the global demethylation associated with normal aging and cancer development [86].

3.2.2. Extragenic Macrosatellite Tandem Repeats and CpG Island Methylation

Extragenic variable number tandem repeats (VNTRs) are categorized based on the number of bases pairs repeated; microsatellite VNTR (1-4 bp), minisatellite VNTR (6-64 bp) and macrosatellite (or megasatellite) VNTR (several kb) [87]. Some VNTRs have a high GC content, and these may be epigenetically modified by CpG island methylation. One example of this is Fragile X-syndrome, where the gene responsible for the disease, *FMR1*, has a typical CpG island and a CGG trinucleotide repeat in its promoter sequence [88]. Expansion of this repeat triggers DNA methylation and subsequent silencing of *FMR1* [89-91]. This hypermethylation of CGG repeats in the *FMR1* promoter is also associated with histone code modifications, such as increased lysine 9 methylation (H3-K9) and decreased lysine 4 methylation (H3-K4) in the histone core protein H3, and 'heterochromatization' by the histone code alterations in Fragile X [92].

Another CpG-VNTR-associated disease is facioscapulohumeral dystrophy (FSHD), a type of muscular degeneration characterized by genetic and/or epigenetic alterations of a 3.3 kb long macrosatellite, D4Z4, located in the telomeric region of chromosome 4q [93]. In the normal population, D4Z4 is present as 11-100 copies on the chromosome 4 telomere and has been shown to repress expression of the upstream genes *ANT1*, *FRG1* and *FRG*2 through the D4Z4-multiprotein repression complex [94]. In FSHD, reduced VNTR length (1 to 10 repeats) causes de-repression due to decreased binding of the D4Z4-multiprotein repression complex [94,95]. D4Z4 has a typical CpG island in its sequence. So the CpG island in D4Z4 is a candidate genomic region that may control upstream genes through epigenetic alterations. In fact, D4Z4 CpG islands are markedly methylated in normal tissues. (Kim and Issa, unpublished observation). Recently, hypomethylation of shortened D4Z4 repeats was observed in FSHD patients [96]. Although the role of genomic and/or epigenomic alterations in human diseases such as FSHD still needs to be elucidated [97], it is clear that epigenetic changes of CpG-VNTRs could play an important role in the development of specific diseases in humans.

3.2.3. Minisatellite Tandem Repeats and DNA Methylation

Heterochromatic regions located in pericentromeres and telomeres have GC-rich sequences, and these regions are also enriched for minisatellite tandem repeats and transposable elements. Previously, it was suggested that centromeric and pericentromeric heterochromatin have an important structural role in sister chromatid cohesion [98] and homologous chromosome pairing [99]. Recently, mini-satellite tandem repeats with high GC-content were implicated in the epigenetic function of heterochromatic regions. Dynamic methylation changes in minisatellites resemble methylation changes seen in global and interspersed repetitive sequences in early embryonic development [100]. The high methylation levels observed after embryonic implantation decrease with advancing age [101].

Further, post-transcriptional histone modifications have been observed in heterochromatic regions [102,103]. Histone-related epigenetic alterations are associated with the silencing of unwanted transcription at unexpressed genes and repetitive DNA sequences in heterochromatic regions [103]. Although the relationship between GC-rich minisatellite methylation and histone modifications has not been well described, DNA methylation and histone modification of GC-rich satellites might be important epigenetic changes.

Simple DNA repeats, including gene duplications, also have a potential role in epigenetic regulation. Gene silencing induced by DNA methylation in DNA repeats has been implicated in repeat-induced point mutations and methylation-induced premeiotic mutations in fungi, as well as in posttranscriptional gene silencing and transcriptional gene silencing in plants [104]. In addition, DNA repeats have recently come into focus as hypothetical triggering sites for DNA methylation and epigenetic gene silencing [104].

DNA repeats in any ectopic and non-physiological location can induce transcriptional suppression through initiation

of heterochromatin formation, and can also trigger the RNAi process via production of double-stranded RNA [105]. Also, generation and accumulation of short heterochromatic RNA from DNA repeats has been reported to be involved in DNA repeat-related RNAi. Short double-stranded RNA in satellite DNA repeats has been reported to signal promoter H3-K9 methylation and subsequent epigenetic modification of the genome. Ultimately, gene expression of nearby genes may be blocked by changes in chromatin structure [106].

An interesting characteristic of some DNA repeats is the presence of inverted segments. These are potent trans-acting inducers of silencing and methylation [104]. Inverted repeats can also cause gene silencing in a methylation-independent manner in Drosophila [107]. Additionally, RNAi triggered by inverted repeats has a potent epigenetic gene-silencing effect in plants, fungi and protozoa [108,109]. In humans, DNA methylation and gene silencing induced by inverse repeats have not been clearly described. Nevertheless, concomitant methylation and silencing of transgenic DNA repeats triggered by a high transgene copy number has suggested the possibility of a DNA repeats/RNAi gene silencing in mammals [110].

4. DNA METHYLATION IN AGING AND AGE-RELATED DISEASES

4.1. Hypomethylation in the Normal Aging Process

Epigenetic changes are frequently observed during the normal aging process [111]. A passage-dependent decrease in total 5-methyl-cytosine was observed in an *in vitro* fibroblast culture [77], and age-related global hypomethylation was also noticed in human lymphocytes [112] and in aging mice [113]. Like chromosomal instability induced by heterochromatin demethylation, genetic instability induced by global hypomethylation has been proposed to be a mechanism for cellular senescence [101]. Therefore, DNA demethylation could decrease *in vitro* life span [114,115] and facilitate premature *in vitro* senescence [116]. However, global hypomethylation has also been observed in proliferating tissues [117], and *Dnmt1* heterozygous mice appear normal for 1 year, even though their methylation level is lower than that of wild-type mice [118]. Thus, the role of global hypomethylation in the aging process needs further clarification.

4.2. Hypermethylation in the Normal Aging Process

As described above, genome-wide methylation of CpG islands decreases with advancing age, although an increase in CpG islands methylation has also been documented in human aging. However, hypermethylation only infrequently develops, but when it does, the promoters of some specific genes are hypermethylated [119].

Estrogen receptor α (*ERα*) promoter hypermethylation was the first unequivocal evidence of age-related hypermethylation in a CpG island in normal tissue [120]. This hypermethylation was detectable in normal colonic tissues adjacent to colonic adenomas and carcinomas, and the increase in the methylation level correlated well with increased age. Age-related hypermethylation was proposed as an epigenetic mechanism for some cancers [121]. In fact, many other genes have been shown to display age-related methylation in aging colonic epithelium. Furthermore, most genes hypermethylated in cancer also show some degree of methylation in aging normal tissue [70]. Thus gene-specific hypermethylation that develops during the aging process could contribute pathologically to the development of age-related diseases, and in particular, cancers.

4.3. Hypermethylation in Age-Related Diseases

Altered physiologic function associated with age-related epigenetic promoter alteration is thought to be a pathological mechanism involved in cancer development. Recently, vascular disease, another typical age-related disease, was shown to be associated with promoter hypermethylation of the estrogen receptors α (*ERα*) and β (*ERβ*). There was a significant increase in hypermethylation of *ERα* and *ERβ* in coronary atherosclerotic plaques [122,123]. Interestingly, a passage-dependent increase in *ERα* and *ERβ* methylation was detected in senescent vascular smooth muscle cells [124] and arterial endothelial cells *in vitro* [123]. Gene-specific-promoter hypermethylation was lower in atherosclerotic tissues than in cancer tissues, though data on this issue remain limited. Future studies investigating epigenetic alterations in age-related diseases as well as in atherosclerosis may address these issues.

4.4. Epigenetic Mosaicism in the Aging Process and Age-Related Diseases

Age-related epigenetic mosaicism has been proposed as a precursor to age-related carcinogenesis [125]. In this model, clonal populations of histologically homogenous tissue reveal the possibility for mosaic epigenetic alterations [126]. The variable and mosaic epigenetic changes in different cells and tissues initially causes subtle expression changes in "primed cells," and subsequent harmful epigenetic and genetic changes accumulate in the predisposed cells leading to cancer through natural selection [125]. The gene and lineage specificity of loss of imprinting of IGF2 observed in Wilm's tumor and intestinal maturation supports the idea of clonal epigenetic variation in cancer development [127,128]. In addition to cancerous tissues, atherosclerotic tissues also show a mosaic methylation pattern in *ERβ*. Coronary atherosclerotic lesions tend to show an all-or-none promoter methylation of the *ERβ* [123]. A similar pattern of *ERβ* methylation was also observed in senescent vascular smooth muscle cells *in vitro* [123]. A mosaic pattern of clonal epigenetic variation is an important model for epigenetic evolution in the aging process and in other age-related diseases where localized proliferation or tissue dysfunction is important.

4.5. Causes of *De Novo* Methylation in Aging and Age-Related Diseases

4.5.1. Transcriptionally Silent Genes and DNA Methylation

The transcriptional activity of a gene can influence epigenetic modifications of that gene. For example, transcriptional suppression of an infecting retrovirus in an embryonic cell developed earlier than *de novo* methylation of the viral sequences did [129,130]. Also on the inactive X chromosome in mammalian embryogenesis, suppression of phosphoglycerate kinase expression developed before promoter methylation of the gene did [131]. Therefore, it has been proposed that active promoters are resistant to DNA methylation; in contrast, promoters of silenced gene are more easily affected by hypermethylation [53,72]. Also, promoter silencing was essential to allow a *de novo* methyla-

tion seed to extensively propagate in the promoter of a gene in prostate cancer [132]. In postmenopausal women, natural depletion of estrogen is a well-known risk factor for age-related diseases like cerebrovascular disease, coronary vascular disease, dementia, and cancer [133]. As described above, an age-related increase in hypermethylation of *ERα* and/or *β* has been reported in normal colon and senescent vascular cells, and it is possible that this methylation results from an initial decline in gene transcription related to a decline in estrogen levels and gene usage. Interestingly, estrogen replacement therapy in postmenopausal women decreased the incidence of colon cancer [134] and prevented recurrence of vascular insults [135,136], though estrogen replacement therapy is now controversial for prevention of age-related diseases [137]. One view of hypermethylation, then, is that it follows transcriptional inactivation and 'locks in' the silenced state for the estrogen receptor, this set up a 'use it or lose it' situation, which could explain the relative ineffectiveness of estrogen replacement therapy in cardiovascular diseases.

4.5.2. Homocysteine and DNA Methylation

Homocysteine-induced vasculopathy was first reported in children with homocystinuria [138]. Recent studies have focused increased homocysteine levels as an independent vascular disease risk factor in the general population [139]. Vascular disturbances possibly related to homocysteine developed even though its level was within the upper normal limit [140].

The molecular mechanism of homocysteine action in the development of vascular pathology has not been completely elucidated. Environmental factors, e.g., decreased folate intake [140], and genetic factors, e.g., methylenetetrahydrofolate reductase polymorphisms [141], could contribute to increasing homocysteine levels. An elevated homocysteine level promotes vascular lesions by inhibiting endothelial cell regeneration and inducing endothelial and smooth muscle cell dysfunction [142].

Epigenetic alterations via hypomethylation changes may an aspect of homocysteine-induced vasculopathy [142]. Homocysteine is a product of a methionine metabolic pathway. Methionine is converted to S-adenosylmethionine (SAM), and S-adenosylhomocysteine (SAH) is produced after a methyl group is enzymatically removed from SAM in transmethyltion reaction. Adenosylation of SAH then produces homocysteine [143]. Homocysteine is remethylated to methionine in the presence of folate and vitamin B12 [143]. Among the metabolites in the metabolic cycle of methionine, SAM is a methyl donor for DNA methylation. With an increase in homocysteine, methionine and SAM levels in the blood decrease, while SAH levels increase [144]. SAM is a universal methyl donor while SAH is an inhibition of DNA methyltransferase. Thus, a decreased SAM/SAH ratio may lead to hypomethylation of DNA [144]. Homocysteine-induced hypomethylation has been observed in liver, brain, testes, lymphocytes, and endothelial cells [142,145,146].

In cancer epigenetics, folate deficiency is correlated with an increased risk of cancers [147], and global DNA hypomethylation is frequently observed in tumor development [148]. However, in another study, hypermethylation in specific genes was observed in a hepatic neoplasm produced in a folate- and methyl-deficient rat, whereas hypomethylation was maintained in control and preneoplastic animals [149]. A similar dual effect (global hypomethylation/gene specific hypermethylation) could also be a result of an increase in homocysteine, and more experiments are needed to clarify potential epigenetic mechanism of homocysteine excess.

4.5.3. Inflammatory Response and DNA Methylation

Inflammation is thought to play an important role in age-related diseases, including cancer. Recently, vascular inflammatory response to injury and lipid peroxidation has also been proposed as an independent risk factor for atherosclerosis [150,151]. Such inflammatory responses can be induced by most of the risk factors for vascular diseases, e.g., hypertension, cigarette smoking, hyperglycemia, and altered lipid profiles [151]. Additionally, evaluation of vascular inflammation has predictive value in determining the risk of vascular accidents [152] and evaluating the effectiveness of preventive medicine [153].

How does inflammation lead to disease? Previous studies have focused in genetic mechanisms, such as reactive oxygen species generation. More recently, there has been interest in epigenetic effects of inflammation. An increasing number of studies are describing inflammation-induced methylation changes, such as in esophagitis [154], gastritis and gastric cancer [155], hepatic cirrhosis [156,157], chronic kidney disease [158], and ulcerative colitis [159]. Ulcerative colitis, in particular, has clinical characteristics of chronic inflammation, rapid cell turnover, and a subsequent risk of colon cancer [160,161]. The unaffected mucosa of patients with severe ulcerative colitis still is shown significant accelration of age-related methylation in genes such as *ERα* and *MYOD* [159]. Hypermethylation of ERα was also observed in cases of esophagitis, which is associated with chronic reflux-induced epithelial damage [154]. These inflammation-induced epigenetic changes may cause abnormal hypermethylation in focal lesions predisposing the affected tissues to further progression towards neoplasia [154,162].

As described above, inflammation is also likely to play a role in the development of atherosclerosis, and hypermethylation of genes, such as *ERα* and *ERβ*, could be a mediation of the effect. The molecular mechanism of the inflammation/methylation remains to be clarified.

5. FUTURE DIRECTIONS

Understanding the molecular basis of aging and age-related disease is one of the most challenging research fields, and advances in this field will require a multidisciplinary approach.

There has been a recent increase in evidence supporting epigenetic regulation of the aging process and age-related diseases. Genome-wide and/or gene-specific promoter methylation changes might be an important epigenetic mechanism relating aging to age-related diseases. However, several issues remain to be clarified for the epigenetic regulation of aging to be understood. Risk factors influencing the development of DNA methylation changes are not well-known in humans. Inflammatory responses [159], transcriptional repression [72,132], and an increase in homocysteine [144] have been reported to initiate DNA methylation changes. However, cardiovascular and other environmental risk fac-

tors, such as hypertension, diabetes, hyperlipidemia, etc., are the main risk factors associated with age-related diseases in humans. Therefore, future efforts should focus on verifying the relationships between cardiovascular risks and age-related epigenetic changes. The elucidation of the environmental contribution to age-related epigenetic changes will increase our understanding of the aging process and help in the design of therapeutic interventions for aging and age-related diseases.

Molecularly, it will also be necessary to scan for age-related-promoter methylation in specific genes. In cancer, promoter methylation changes and the resulting repression of specific gene expression have been observed [163]. In the aging process, although promoter hypermethylation in some genes has been observed, the molecular significance of these changes is not well understood. Recent whole genome methylation scans have been developed [164,165], which will facilitate the search for methylated genes and related expression changes. However, caution is needed when analyzing such data because age-related DNA methylation changes are usually less frequent than the changes that occur in cancer tissues [123]. Therefore, very sensitive and specific methylation detection systems will be necessary to identify methylation changes in aged tissues.

ACKNOWLEDGEMENTS

This work was financially supported by Regional Research and Development Cluster Project (B0009735) Funded by the Ministry of Knowledge Economy (MKE) of Korea.

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Received: May 08, 2008 Revised: July 21, 2008 Accepted: July 28, 2008