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Genome **Biology**

RESEARCH HIGHLIGHT

The aging epigenome: DNA methylation from the cradle to the grave

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Abstract

Whole-genome methylation analysis of newborns and centenarians reveals widespread epigenetic alterations, and provides new insight into age-related methylation pattern changes.

There is growing evidence that epigenetic mechanisms play a crucial role in regulating a variety of biological processes, including aging. In addition to histone modifications, DNA methylation is a key epigenetic mechanism that controls gene expression and thereby alters cellular phenotypes. DNA methylation occurs at the carbon-5 position of cytosines located next to a guanosine (CpG dinucleotides). Although the total number of CpG dinucleotides is reduced in the human genome, CpG-rich sequences (CpG islands) are present and often located in promoter regions. More than half of all human genes harbor a CpG island, which is usually unmethylated. However, hypermethylation of specific CpG islands has been demonstrated in a wide range of biological conditions and shows a strong association with decreased gene expression. In addition, DNA methylation marks located outside of promoter regions (gene bodies, for example) can also regulate gene expression and are generating increasing interest [1]. A recent publication has now shed new light on age-related epigenetic changes by studying the methylomes of a newborn and a centenarian [2].

Age-related methylation changes

Aging is a multifactorial process that results in a progressive loss of regenerative capacity and tissue functionality. The molecular changes associated with aging are multilayered and are only beginning to be understood. One of the first studies that analyzed the DNA methylation changes during aging in detail suggested that the global methylation pattern of monozygotic twins becomes more variable during their lifetime, thus indicating age-related epigenetic variation [3].

More recently, the development of array-based technologies has allowed the design of more detailed studies to further characterize age-dependent DNA methylation changes. For example, methylation profiling with an early array platform (1,505 CpG sites representing more than 800 cancer-associated genes) suggested that age-dependent hypermethylation occurs in various human tissues and at well-defined CpGs within CpG-island-associated promoters [4]. In addition, hypomethylated CpGs were also identified, predominantly in promoter regions with low CpG content, thus illustrating the complexity of age-related methylation changes [4]. Additional insight came from a second generation of array experiments that provided a more unbiased genome coverage (27,578 CpG sites representing about 14,000 genes). For example, methylation profiling of skin samples obtained from young and old volunteers identified a small set of gene loci that were hypermethylated in old skin [5]. Importantly, this study also demonstrated that the array-predicted methylation changes at single CpG dinucleotides actually extend over entire promoter regions and thus resemble epigenetic mutations [5].

Additional studies showed that age-associated differentially methylated regions were conserved in several tissues, indicating that age-dependent methylation changes are not acquired randomly [6,7]. The specificity of age-related methylation changes was further supported by the observation that hypermethylation occurs preferentially at bivalent chromatin domains, which suggests the mechanistic involvement of chromatin-modifying factors in this process [6,7].

Newborn and centenarian methylomes

While these initial studies strongly suggested the existence of age-related methylation changes, they were also curtailed by the limited analytical sensitivity and resolution power of the array platforms that they used. The human genome contains about 20 million CpG dinucleotides on each strand and there are presently no

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defined subsets of CpGs with clear prognostic relevance. This has fueled the development of sequencing technologies that permit the generation and analysis of whole-genome methylation maps at single-base resolution [8].

A recent publication by Manel Esteller and colleagues [2] now sheds new light on age-related epigenetic changes by using whole-genome sequencing approaches to study the methylomes of a newborn and a centenarian. The authors obtained about 100 Gb of DNA sequence from purified CD4+ T cells, which corresponded to a combined genome coverage of 26-fold. The comparison of the two individual data sets revealed three key differences. First, the centenarian methylome showed a pronounced hypomethylation relative to the newborn that affected about 500,000 CpG dinucleotides. This effect decreased the average CpG methylation levels from 81% in the newborn to 73% in the centenarian, and it was observed throughout the genome and for all genomic compartments, such as promoters, gene bodies and intergenic regions. Second, the centenarian methylome showed a loss of DNA methylation homogeneity. In the newborn, DNA methylation levels of neighboring CpG dinucleotides were often similar. In the centenarian, however, local DNA methylation patterns appeared more heterogeneous, and this can be related to the hypomethylation of specific CpG dinucleotides. Third, a detailed analysis of regulatory regions showed that age-related hypomethylation was particularly pronounced in CpG-poor promoters and in genes that show tissue-specific gene expression. Hypomethylation of these regions in centenarians was also associated with moderately higher levels of gene expression.

These key findings were validated by array-based methylation profiling of a larger set of unfractionated blood samples (19 newborns, 19 centenarians). Further validation was obtained by array-based methylation profiling of blood samples from 19 middle-aged donors. Interestingly, the array-based analysis also confirmed another observation made in the sequenced samples: CpG island promoters, which are regulatory regions with a strong impact on gene expression, can become more methylated with age. These results are in agreement with earlier reports that have described CpG island promoter hypermethylation during human aging [5-7].

Perspectives and mechanistic insight

The results from Esteller and colleagues [2] also represent an important starting point for future research. The focus of the study on extreme points of the human lifespan renders the results somewhat susceptible to biological factors not related to aging. For example, T cells from newborns are still immature and it has been shown that the maturation of hematopoietic cells is associated with hypomethylation of many differentiation-associated genes [9,10]. Similarly, reductions in DNA methylation pattern homogeneity, as observed in the centenarian sample, could be indicative of an increased biological sample heterogeneity, as biological differences within CD4+ subpopulations could become more pronounced with advanced age. To resolve these issues, it will be important to confirm the described methylation changes in other tissues.

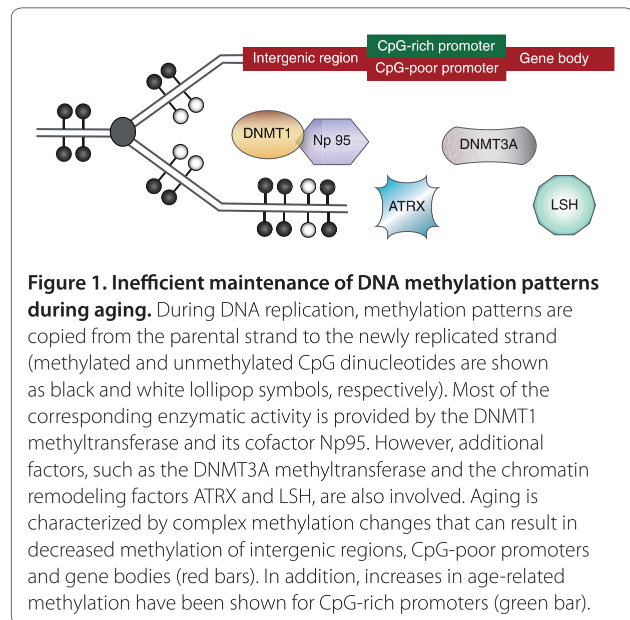

Figure 1. Inefficient maintenance of DNA methylation patterns during aging. During DNA replication, methylation patterns are copied from the parental strand to the newly replicated strand (methylated and unmethylated CpG dinucleotides are shown as black and white lollipop symbols, respectively). Most of the corresponding enzymatic activity is provided by the DNMT1 methyltransferase and its cofactor Np95. However, additional factors, such as the DNMT3A methyltransferase and the chromatin remodeling factors ATRX and LSH, are also involved. Aging is characterized by complex methylation changes that can result in decreased methylation of intergenic regions, CpG-poor promoters and gene bodies (red bars). In addition, increases in age-related methylation have been shown for CpG-rich promoters (green bar).

It is also likely that the functional consequences of age-related methylation changes will come more into focus in future studies. Initial pathway analyses of genes that are differentially methylated in newborns and in centenarians [2] were not particularly revealing and we still lack a clear understanding of the effects that age-related methylation changes have on the transcriptome. A systematic characterization of transcription patterns by transcriptome sequencing should provide additional insight into the effects of age-related methylation changes on gene expression and on the aging phenotype.

Finally, it will be very important to elucidate the molecular mechanisms that underlie age-related methylation changes. It is conceivable that the observed changes can be interpreted to reflect an inefficient maintenance of methylation patterns during aging. The maintenance of methylation patterns is a complex process involving many factors (Figure 1). While the DNMT1 methyltransferase catalyzes most of the corresponding enzymatic activity, the process is tightly regulated by cofactors such as Np95. In addition, the *de novo* methyltransferases DNMT3A and DNMT3B do not only have the capacity to establish new methylation marks, but also contribute to methylation maintenance. ATRX, LSH and other chromatin remodeling factors are also known to play an

important role in the faithful maintenance of DNA methylation patterns. Altogether, these factors form a complex regulatory network that can affect genomic methylation patterns in several ways (Figure 1). A more detailed understanding of these mechanisms will allow us to better understand age-related methylation changes and might also facilitate the development of specific intervention strategies.

Competing interests

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