

## NEWS AND VIEWS

# DNA methylome: Unveiling your biological age

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Hannum and colleagues performed DNA methylation sequencing to examine the relationship between DNA methylome and aging rate. Notably, they succeeded in building a quantitative and reproducible model based on the epigenetic bio-markers to predict aging rate with high accuracy. This progress enlightens us in many aspects particularly in applying this novel set of bio-markers on studying the mechanism of aging rate using adult tissue-specific stem cells, building up a potential quantitative model to explore the mechanism for other epigenetic factors like non-coding RNA, and understanding the principle and mechanism of 3D chromatin structure in epigenetic modulation.

Aging and aging related diseases, characterized by gradual deterioration of functional capabilities and ultimate death, have been a field attracting intense interest over centuries. Epigenetics is the study of heritable phenotypic changes that regulate genetic information without DNA sequence alterations including DNA methylation, histone modifications, chromatin remodeling and non-coding RNAs. Interestingly, a growing body of evidence supported a strong correlation between aging and epigenetic regulation. It was suggested that epigenetic mechanism played a critical role in aging and aging related diseases and was even perceived as a candidate hallmark of aging (Lopez-Otin et al., 2013). In particular, alterations in DNA methylation pattern have been reported to be linked with chronological aging process in human studies. For instance, a longitudinal study revealed a global loss of DNA methylation particularly in the repetitive elements (such as Alu) during aging (Bollati et al., 2009). Subsequent study further discovered

that DNA was hypermethylated located near tissue-specific CpG islands; whereas hypomethylation was significantly associated with none CpG-island region during aging by looking at the methylation profiles (Christensen et al., 2009). A more detailed study on methylome of CD4<sup>+</sup> T cells in newborns and centenarians confirmed Christensen et al.'s finding and added that there was a less DNA methylation content and a reduced correlation of neighboring CpG methylation status in centenarians relative to newborns (Heyn et al., 2012). Moreover, Gentilini et al.'s study indicated a delay of global methylation decrease in the centenarians' offspring in comparison with those of non-lived parents. More interestingly, those two groups also exhibited different methylation profiles in genes involved in RNA/DNA biosynthesis, metabolism, and signal transmission (Gentilini et al., 2012). The above evidence suggests that changes to DNA methylation landscape were associated with aging. Nevertheless, systematic description and quantitative measurement

of DNA methylome as well as how it affects aging are yet-to-be revealed.

Hannum et al. made noteworthy progress in addressing these questions. In order to find methylation markers related to aging, the group built a quantitative model among 485,577 CpG markers to describe the aging rate of individuals. They found 15% of these markers were related to aging, and then they compared their result with Heyn et al.'s study and screened 53,670 sites. To pick out more relevant aging-related markers, the group applied predictive model of Elastic Net (Zou and Hastie, 2005) along with bootstrap approaches, and identified 71 methylation markers. Then, the group built their aging model with these 71 markers and employed various tissue samples to validate it. The authors specifically noted that nearly all these markers are located nearby or within genes that have known functions in aging or aging-related diseases. The group made a thorough analysis of these markers on both gene and methylation profiles to identify the relationship between these

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aging-related methylation markers and aging-related genes.

It is worthwhile to highlight that the group is the first to succeed in building a quantitative, strong and reproducible epigenetic model as biomarker irrelevant of tissue-specificity and gender variants to predict biological aging rate with high accuracy. In addition to aging rate prediction, this model can also be applied to identify individuals who do not follow the expectation. It is worth noting that the technical improvement made in the experimental design. The study chose a large cohort of individuals (656) with a broad age range from 19 to 101 and also applied the finding to another large cohort of individuals. Their key finding obtained from blood sample was also validated in various tissue samples. Notably, they were also the first to reveal the intertwined relationship between genes and aging methylome. They confirmed the previous hypothesis that certain genetic variants (such as DNA repair related genes) broadly influenced aging methylome and disclosed that age-associated methylome changes could be an indicator of change in gene expression patterns. The authors also provided a well-grounded evidence and explanation on epigenetic drift, an interesting concept which premises that individuals share similar methylome at younger age; as accumulating changes during aging, the methylomes become divergent.

Though the study did not confirm the causal link between DNA methylome and aging, Hannum et al.'s study inspires to explore these questions in several aspects. To start with, this quantitative model could be perceived as a promising aging biomarker to study the effect of environmental cues on aging and aging-related diseases. Particularly, this quantitative model highly correlates with functional gene expression patterns. Telomere length has been demonstrated as a successful biomarker of aging in human (Harley et al., 1990), but it is far from an ideal biomarker due to heterogeneity, mosaicism as well as difference on proliferating capability, especially when it comes to adult stem

cells. Adult stem cells including skin stem cell (SSC), hematopoietic stem cells (HSC), neural stem cells (NSC), mammary stem cells (MaSC) and mesenchymal stem cells (MSC) etc., hold the capability to self-renew and differentiate thus contributing to the tissue/organ regeneration as well as mediating homeostasis (Rossi et al., 2008). Recent evidence showed that HSC decline was irrespective of telomere length during physiological aging and experimentally forced proliferation (Beerman et al., 2013). Rather, there was a correlation between alterations of DNA methylation and HSC lineage potential and aging of HSC leads to DNA hypermethylation (Beerman et al., 2013). As the relationship between aging and adult stem cell decline has long been studied and evidence by far suggests a mutual cause and effect relationship (Kirkwood, 2004; Sahin and Depinho, 2010; Doles et al., 2012; Jane-Wit and Chun, 2012; Beerman et al., 2013). If aging-associated methylome change plays a decisive role in aging-related tissue stem cell decline, a reliable biomarker is extremely in need to further study underlying mechanism. Hannum et al.'s quantitative model can be employed in such system to obtain and analyze the complete genome-wide methylation profile of adult stem cell, and to identify aged stem cells. It also offers a new platform to study the underlying mechanism of stem cell decline and aging.

Hannum et al.'s finding highlights the significance of epigenetic mechanism on aging, thus prompting us to construct a bigger epigenome picture related to aging. D'Aquila et al. assumed that the epigenetic reason for aging was the time-related instability of chromatin 3D structure caused by epigenetic regulation and they stated that this instability could also be caused by histone modifications, non-coding RNA (ncRNA), and components from mitochondria as aging-related epigenetic modulators (D'Aquila et al., 2013). Based on Hannum et al.'s work, we can apply similar techniques of sensitive sequencing and data mining on multiple epigenetic mechanisms including histone methylation and ncRNAs, to construct a more

delicate model to predict aging. Similarly, such model can also be used as biomarker for aging study in model systems like human induced pluripotent stem cells and adult stem cells etc. and be applied to study mechanisms and establish a potential link between epigenetic modulation, genomic instability, and aging (Liu et al., 2011a, 2011b; Han and Brunet, 2012; Liu et al., 2012a, 2012b; Inukai and Slack, 2013). By exploring these relationships, we can build an aging-related epigenetic network so that a better understanding and theoretical studies of epigenetic factors essential for aging can be achieved. With regards to regenerative medicine, as corrections on alterations of genomic sequences have not been proved efficient so far, we hope that epigenetic rejuvenation would be a better solution for aging and aging-related diseases.

## FOOTNOTES

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