Epigenetic Aging: More Than Just a Clock When It Comes to Cancer

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ABSTRACT

The incidence of cancer, adjusted for secular trends, is directly related to age, and advanced chronologic age is one of the most significant risk factors for cancer. Organismal aging is associated with changes at the molecular, cellular, and tissue levels and is affected by both genetic and environmental factors. The specific mechanisms through which these age-associated molecular changes contribute to the increased risk of aging-related disease, such as cancer, are incompletely understood. DNA methylation, a prominent epigenetic mark, also changes over a lifetime as part of an "epigenetic aging" process. Here, we give an update and review of epigenetic aging, in particular, the phenomena of epigenetic drift and epigenetic clock, with regard to its implication in cancer etiology. We discuss the discovery of the DNA methylation–based biomarkers for biological tissue age and the construction of various epigenetic age estimators for human clinical outcomes and health/life span. Recent studies in various types of cancer point to the significance of epigenetic aging in tumorigenesis and its potential use for cancer risk prediction. Future studies are needed to assess the potential clinical impact of strategies focused on lowering cancer risk by preventing premature aging or promoting healthy aging.

Aging and Age-Related Disease

The average human lifespan has dramatically increased over the last century (1). By 2035 it is estimated that there will be 78 million people >65 years of age in the United States (2). The growth of the aging U.S. population has drawn increased attention to the process of aging and factors that mediate age-related declines in health and promote age-related disease. The molecular, cellular, and physiologic changes associated with aging are myriad and include, among others, cellular senescence, the accumulation of genetic alterations, onset of low-grade inflammation ("inflammaging"), protein misfolding, epigenetic alterations, and cumulative oxidative stress damage (3, 4). These processes may induce age-related tissue decline and increase disease risk by influencing the proliferative potential of stem cell pools, impairing immune surveillance, and altering the expression of oncogenes and tumor suppressor genes (5). The accumulation of epigenetic alterations in aging cells reflects intrinsic and extrinsic processes that progressively perturb the epigenetic state of aging cells, and a subset of the alterations show drift- or clock-like behavior. Epigenetic age, as estimated by drift/clock CpG dinucleotides, is receiving increased attention because it appears to be a robust marker of tissue age and to hold promise for improving the risk prediction of age-related diseases.

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Cancer epigenetics and its relation to aging

It is well established that genome-wide changes in the methylation status of CpG dinucleotides, a well-studied epigenetic feature of the human genome, occur during the process of cancer formation. Some cancers even have unique methylomes that define distinct molecular subtype of the cancer. In colorectal cancer, a hypermethylator phenotype called the CpG island methylator phenotype (CIMP) that is seen predominantly in the elderly and in the right colon, can be identified in approximately 15% of cancers (6–9). Molecular subtypes based on DNA methylation (DNAm) have been identified in many cancer types including gliomas (10, 11), gastric cancer (12) and its precursor intestinal metaplasia (13), acute myeloid leukemia (14), and recently in esophageal adenocarcinoma (15).

The epigenetic aging process is of particular relevance to our understanding of cancer in light of multiple studies on the aberrant DNAm changes associated with aging and the apparent relationship of epigenetic aging to tumorigenesis (16–23). Recently, genome wide epigenetic studies have revealed particular epigenomic features shared between aging and cancer. First, gain of DNAm occurs preferentially at CpG dense promoter regions, upstream of genes that are important during development and that are implicated as tumor suppressors in cancer (20, 22, 24). Notably, some sequences that undergo methylation alterations are associated with polycomb group 2 (PCG2) binding sites and are transcriptionally repressed in adult stem cells (22, 25). Conversely, a pronounced and consistent pattern of DNAm loss is observed at lamina-associated, partially-methylated domains in primary cancer samples, as well as in aged tissue (26, 27). The gradual loss of DNAm may be due to the low fidelity of the DNA methyltransferase (DNMT) coupled to DNA replication during cell division or to impaired active demethylation mechanisms [e.g., ten-eleven translocation (TET)]. The DNAm loss process likely occurs in aging tissue as well as cancer, although it appears to be aberrant in cancer. In the next sections, we will review our current understanding of DNAm changes as part of an "epigenetic aging" process, and discuss their implications in cancer etiology.

The difference between chronologic age and biological age

Chronologic age, by definition, is a measure of the number of years an individual has lived. Yet, it is well appreciated that people age physiologically at different rates, and individuals of the same

chronologic age may have different biological ages. Thus, an individual's biological age, a proxy for an individual's overall physiologic performance status and health status, can vary from his/her chronologic age (28), displaying faster (acceleration) or slower (deceleration) aging rate, compared with normal aging (**Fig. 1**). The deviance of one's biological age from chronologic age, being younger or older, reflects the biophysiologic aging status of the individual. Thus, using biological age should be a more accurate way to predict an individuals' health/disease status and their aging process compared with his/her chronologic age. A major challenge in aging research is the identification of accurate measures of an individual's biologic age.

Biological tissue age and DNAmethylation

An ideal biological age estimator is considered to be a useful biomarker if it correlates with chronologic age but, importantly, also with additional information on an individual's risk for age-related conditions. Numerous physiologic, functional, cellular, and molecular features have been proposed as biological age predictors over the last several decades, but few of them have been shown to be robust or precise (29). Recently, a number of promising biomarkers of biological age have been identified, including biological age estimators based on DNAm, telomere length, transcriptomics, proteomics, metabolomics, and composite biomarker panels (29, 30). Among them, two types of DNAm-based age predictors, referred to as epigenetic clocks and as epigenetic drift, have been demonstrated to perform well as biological age predictors in multiple studies (23, 31–33).

Epigenetic clocks are developed using regression methods to select specific sets of DNAm markers (CpG dinucleotides) that provide reliable estimates of an individual's chronologic age, or an individual's likelihood to have an age-related condition (**Fig. 2**). As individuals age, DNAm patterns undergo characteristic age-related changes presumably secondary to effects caused by the human microbiome, lifestyle, and environmental factors, and more directly through epigenetic drift associated with errors in maintaining DNAm patterns.

Epigenetic drift describes the accumulation of errors that may result from the imperfect fidelity of DNMT enzymes that copy DNAm patterns from parental DNA strands onto newly synthesized daughter strands during DNA replication and from errors resulting from faulty DNA damage repair. Drift may also result from an active demethylation process during ageing via TET enzymes to remove the methyl groups from the deoxycytosine. Drift rates may differ between tissues depending on cell proliferation rates, inflammation, and disease-related processes. CpG dinucleotides or regions that are initially unmethylated generally tend to drift upward to higher methylation values. Although the mean DNAm level of a specific CpG [or CpG island (CGI)] across a group of cells may drift either upward or downward with age, the variance of the DNAm levels between tissue samples and between individuals generally increases with age.

The discovery of molecular alterations as accurate markers of biological age is of further interest because clocks that tick via biological processes would be predicted to increase cellular entropy, which may ultimately interfere with homeostatic regulation. Such alterations may therefore play a functional role in the aging process and in cancer development beyond their role as autonomous clocks.

Epigenetic clocks

The concept of an epigenetic clock refers to a calibrated set of CpGs, which are used to estimate chronologic or tissue age. In a series of bioinformatic studies (34–37), genome-wide DNAm data were derived from HumanMethylation 450 methylation beadchip arrays (Illuimina) for blood, brain, and various other tissue types. These studies found strong correlations between methylomic changes and the chronologic age of the sample donors for specific CpG probes using regularized elastic net regressions. The derived methylated CpG clock models were shown to be surprisingly accurate, allowing an estimation of a person's age within a range of just a few years (35, 36). Some of these DNAm age predictors may be tissue specific whereas others are tissue agnostic. For example, the Hannum clock composed of 71 CpGs was derived using blood samples (35). Its performance in other tissue types awaits further study. In contrast, the multi-tissue age predictor consisting of 353 CpGs created by Horvath has been shown to accurately estimate chronologic age consistently across several distinct tissue types, possibly owing to its derivation being based on large datasets covering over 50 tissue and cell types (36).

The mechanism(s) that drive this epigenetic clock phenomenon are not clear, but they do not appear to be linked to cell proliferation, as these clocks appear to operate in nonproliferating tissue (e.g., brain) as well as proliferating tissue (e.g., peripheral blood leukocytes; ref. 38). Thus, it is unlikely that the Horvath clock constitutes a mitotic clock measuring the number of cell divisions occurring in the stem cells. In spite of the lack of a deeper understanding of what makes these clocks "tick," this work precipitated further investigation into epigenetic clock phenomena that associate with age-related increases in cancer risk and risks of other age-related diseases or physiologic declines. A prominent example of such a clock is the epigenetic timer of cancer (epiTOC) clock (33). Unlike the Horvath or Hannum clocks, which attempt to predict chronologic age in absolute values, epiTOC was constructed as a DNAm-based mitotic clock that used a relative score estimated from a set of 385 CpGs mapping to the promoters of polycomb group target genes (33). The methylation status of these selected CpGs is zero (unmethylated) in human fetal tissues and increases with chronologic age. Although originally constructed from whole blood samples, the epiTOC was validated in nine normal tissue types to correlate with intrinsic stem-cell division rate estimates. Furthermore, the epiTOC appears to tick faster in cancer tissue compared with age-matched normal tissue from 15 The Cancer Genome Atlas (TCGA) cancer types, reflecting the universal hallmark behavior of increased cell proliferation seen in cancer (33). Unlike the Horvath clock, the epiTOC exhibits age acceleration in the at-risk normal buccal tissue from smokers compared with nonsmokers, and in normal breast tissue from patients with cancer compared with healthy women, making it a unique biological clock for estimating cancer risk.

Epigenetic drift

In contrast to epigenetic clocks, epigenetic drift refers to a stochastic process that involves both gains and losses of the methylation state of CpG dinucleotides over time. The rates of epigenetic drift of affected CpG dinucleotides may be dependent on their basal methylation states and on the methylation state and nucleotide composition of flanking sequences (39). Epigenetic drift is a genome-wide process and appears to be a collective phenomenon that correlates strongly with chronologic age and appears to operate in a tissue-specific manner (40–43). Drift occurs when the original methylation states of CpG dinucleotides are altered, presumably as a result of an error during the transfer of the epigenetic marks to the daughter strand following DNA replication. It is believed to be a consequence of the relatively low fidelity of the DNMTs (44–48). Epigenetic drift rates appear to be influenced by interactions between methylated CpGs in CGIs, which are regions of DNA that have a higher CpG content than seen in the genome on average. As a consequence, CGI methylation states generally provide more stable estimates of epigenetic drift compared with individual CpG methylation states (41, 46). Of note, with regards to epigenetic

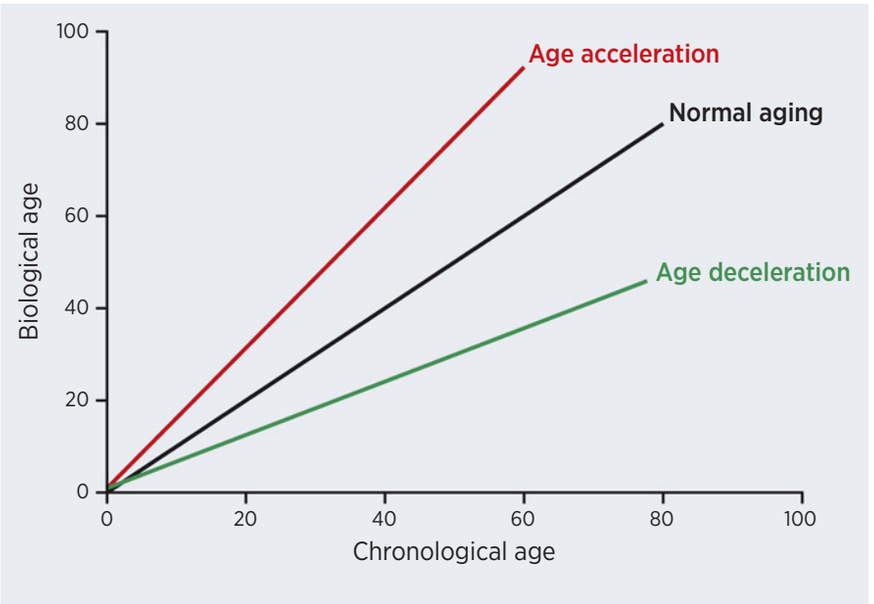

Figure 1.
Epigenetic clocks allow comparison of an individual's chronologic age to his or her biological age, as calculated on the basis of DNAm clock CpGs. Different individuals appear to have different epigenetic aging rates, with their biological (or tissue) age accelerated or decelerated compared with their chronologic age, which may be secondary to intrinsic individual differences (e.g., due to genetics) and to extrinsic epigenetic modifiers (e.g., lifestyle, microbiome, nutrition, and other exposures).

drift, either the collective methylation drift rate (a first order process) or the increase in variance of CpG methylation levels with age (a second order process) may be used as a measure of tissue age (35, 41).

Unlike epigenetic chronologic clocks (i.e., Horvath or Hannum clock), epigenetic drift appears to track biological tissue aging. When drift is caused by sporadic gains of CpG methylation at positions that are predominantly unmethylated, such as CpGs on islands overlapping gene promoters, it is likely a potential marker for the number of overall cell divisions or cell turnover rate in a tissue. Thus, one important application of epigenetic drift may be to "time" the natural history of a specific disease and its progression through early and late states, thereby allowing a determination of the age (or sojourn time) of certain tissue abnormalities (40). This information could be useful for optimally determining when cancer prevention interventions should be initiated. One prominent example of such an application has been recently published and provides a unique perspective on our understanding of the cancer initiation and progression sequence in the colon (41). This is of potential clinical significance because, despite significant advances in our understanding of the molecular pathology of colorectal cancer, little is known about the dynamics of the carcinogenic process and when an adenoma that is ultimately destined to become a cancer first forms within a person's lifetime.

In a recent study, our team sought to better understand the dynamics and role of epigenetic drift in the colon and rectum as an indicator of tissue aging and as a way to more accurately estimate the sojourn time of the adenoma-carcinoma sequence (41). This analysis showed that age-related epigenetic drift is a genome-wide phenomenon, which occurs in normal colorectal tissues and that also appears to progress at an accelerated pace in colorectal neoplasia. We also found that epigenetic drift has increased variance (a second-order drift process) in colorectal cancer compared with normal colon tissue. Importantly, differences in age-related drift between normal and neoplastic tissues were shown to be broadly consistent with colorectal cancer model predictions of long-duration (and highly variable) adenoma-carcinoma sojourn times. Interestingly, we found an unexpectedly early age of onset of adenoma initiation based on the estimated sojourn times. For example, for patients who develop cancer around age 80, the most likely initiation time for the founder adenoma cell is predicted to be very early in life, roughly between the ages 15 and 20 years (see Supplementary Fig S1 in SI of Luebeck and colleagues; ref. 41). This unexpected and provocative finding suggests that the optimal age-range for prevention of colorectal cancer may be in adolescence and early adulthood (and ideally through lifelong) dietary and lifestyle interventions.

Similar results were obtained in a parallel study of differential methylomic drift in Barrett's esophagus (BE), the only known precursor lesion for esophageal adenocarcinoma (discussed in more detail later; ref. 42). Using the methylome of normal distal esophageal tissue as a reference, we found that coordinated epigenetic drift was widespread in BE genomes, and that the amount of drift was highly variable between individuals, suggesting significant differences in BE tissue age. Furthermore, in a cohort of 22 familial patients with BE (i.e., patients who had a first- or second-degree relative with long-segment BE, adenocarcinoma of the esophagus, or adenocarcinoma of the gastroesophageal junction), we found significantly earlier mean BE onset times compared with nonfamilial cases, suggesting that BE arises significantly earlier in life for patients with familial BE compared with nonfamilial BE cases, possibly because of a heritable predisposition for BE metaplasia.

Epigenetic drift versus epigenetic clocks

Although epigenetic drift relates to a biological process that changes the DNA methylome with age via stochastic gains or losses of DNAm, epigenetic clocks are constructed through a regularized selection of a set of markers to estimate chronologic age. Often with epigenetic drift, the focus is on identifying differences in locus-specific drift rates between tissue types and with disease progression, whereas an epigenetic clock can be optimized to provide a tissue independent measure of chronologic age. An epigenetic clock may also be used to predict a variety of age-related outcomes including all-cause mortality, cancer, physical functioning, and Alzheimer disease (23). The decision on which clock construct is most appropriate for the study of the heterogeneity between people with regards to an age-related disease

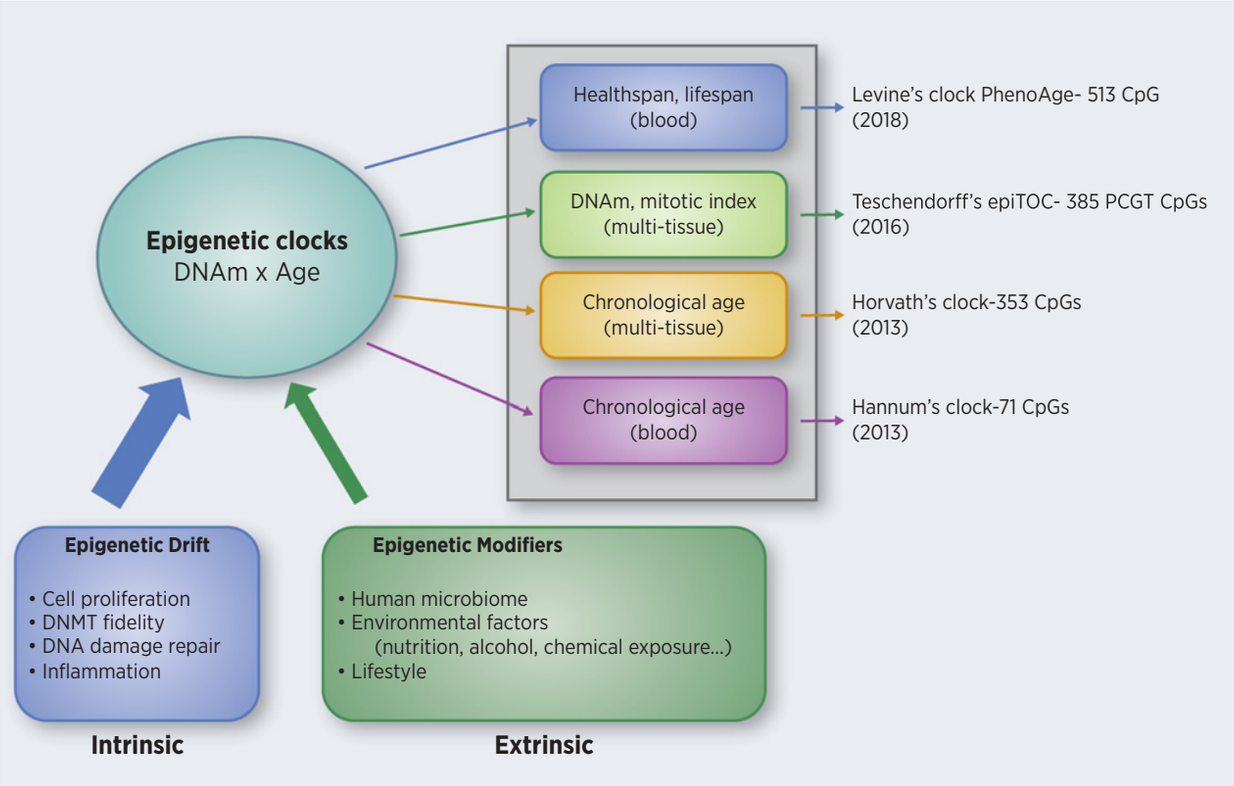

Figure 2.

Age-related alterations in DNAm accumulate in normal tissues and may be used to develop epigenetic clocks to estimate an individual's age or risk for age-related disease. Epigenetic drift may be linked to a variety of biological processes (cell proliferation, inflammation, oxidative stress and DNA repair, one-carbon (1C) metabolism, among others) and DNA damage repair through imperfect fidelity of DNMT enzymes that copy DNAm patterns during cell division. Extrinsic modifiers of the epigenome include the microbiome, lifestyle, and environmental factors. Epigenetic clocks are typically developed using elastic net or other regression methods to link specific CpG dinucleotides on DNA to an individual's chronologic age [Hannum's 2013 clock with 71 CpGs (35) and Horvath's 2013 clock with 353 CpGs (36), to mitotic index in normal tissue and cancers based on DNAm of polycomb group target (PCGT) genes (epiTOC using 385 PCGT CpGs), and to life/health span based on clinical markers of disease (Levine's 2018 PhenoAge clock using 513 CpGs; ref. 23)].

likely depends on the underlying biological processes and pathogenesis of the disease under study. Epigenetic clock constructs that rely on regularized regressions of individual health scores associated with lifestyle factors, environmental exposures, and other factors on DNAm may reveal important mechanisms that contribute to disease progression, and may yield quantitative prognostic biomarkers (23). For cancer and its precursors, chronologic epigenetic predictors such as Horvath's clock are likely not ideal for measuring biological age in cancer, because for at least for some cancer-types "biological age" as measured with Horvath's clock is not accelerated (49). Indeed, using 5621 DNAm profiles of 25 TCGA cancer types, Lin and colleagues showed no correlation between age-associated DNAm and the chronologic age of cancer samples (50). On the basis of the recognition that increased cell proliferation in association with clonal growth is a hallmark feature of many cancers, a biological clock such as the epiTOC, or epigenetic drift that is linked to the overall number of stem cell divisions, may be informative in at least two ways: (i) to provide an estimate of cumulative stem cell divisions and therefore accumulation of DNA sequence mutations, and (ii) to determine changes in gene expression associated with age-related monotonic increases or decreases in DNAm at gene regulatory sites, for example, gene promoters, enhancers, silencers, and transposable elements. Although epigenetic drift can be understood in terms of errors in DNAm maintenance during DNA replication, as described in ref. 46, the mechanisms underlying phenotypic epigenetic clocks still need to be determined and experimentally tested. Importantly, it also remains unclear to what degree epigenetic clocks rely on epigenetic drift at regulatory elements and to what degree they represent a linearization of complex epigenetic changes over time.

Putative molecular mechanisms of the epigenetic aging process

The molecular mechanisms responsible for the epigenetic aging process remain to be confidently determined, but several putative candidate mechanisms have been identified. The first candidate mechanism is the error prone nature of the DNMT enzymes that do not copy with 100% fidelity the DNAm patterns from parent DNA strand to daughter DNA strand. Consistent with this mechanism as a cause of cancer related to DNAm alterations, hypomethylated CpG dinucleotide rich regions (i.e., CGIs) have been shown to undergo epigenetic drift because of sporadic *de novo* methylation errors that occur when tissue stem cells replicate their genome hundreds to thousands of times during an individual's lifetime (51–54). A second mechanism that is

concurrently in operation and that leads to aberrant DNAm is related to the base excision repair DNA repair system. It is postulated that hypermethylated CpG regions may gradually or quickly lose their methylation marks, either passively when DNAm maintenance is compromised during DNA replication or actively via TET enzymes that oxidize 5-methylcytosines and promote the locus-specific reversal of DNAm through base excision repair (55). The relative contributions of passive and active processes on epigenetic aging are not known but both mechanisms likely lead to the aberrant DNAm of candidate tumor suppressor genes and oncogenes. Although DNMTs are generally active only during cell proliferation, epigenetic clocks show age-progression in nonproliferating tissues (37, 56), and recent studies provide evidence of dynamic DNA modifications in non-dividing neurons (57). These results strongly suggest processes involved in active demethylation in cancers (58, 59). However, despite the plausibility of TET affecting epigenetic clock behavior, there is little direct evidence demonstrating the role of active demethylation, other than through TET enzymes or base excision repair, in epigenetic aging processes (60, 61).

Determining the mechanisms behind epigenetic clocks is of more than academic importance because it could inform us how they relate to other age-related cellular processes such as senescence, mutation accumulation, loss of mitochondrial function, and epigenetic silencing and cancer (62–64). For example, because DNA maintenance enzymes (*DNMT*) act in concert with the DNA replication fork (65–67), higher drift rates may either reflect higher rates of cell cycling or a higher rate of methylation errors. The importance of distinguishing the roles of cell-cycle frequency versus methylation error rates may be appreciated when one considers that the aberrant DNAm resulting from low fidelity DNMT activity can globally contribute to increased genomic alterations and epigenetic alterations, whereas in contrast, an increased rate of *de novo* methylation errors likely would mainly contribute to cancer formation via altering specific cancer driver genes, like *MLH1*, because these would require clonal expansion events to be detectable (68).

Beyond intrinsic cellular mechanisms, a variety of studies in ex vivo tissue culture systems and animal models have demonstrated that repeated exposure to environmental factors, such as reactive oxygen species from oxidative stress, dietary factors (e.g. genestein, curcumin) can also induce DNAm alterations, raising the possibility that epigenetic aging may also result from cumulative lifetime exposures affecting these factors (69–71).

Epigenetic Aging and Cancer Risk: Knowledge Gaps

Does epigenetic aging affect cancer risk?

Although the Horvath and Hannum clocks are highly correlated with the chronologic age of the individual, they also show considerable variability around the population mean, which suggests that they may also measure a component of the biological age of the individual (35, 36). This raises the question as to whether there is a mechanism underlying the epigenetic alterations that drive the divergence between chronologic age and biological age. If so, then it may be possible that inter-individual differences in susceptibility to cancer risk are significantly associated with differences in biological aging and the predicted biological age as measured by epigenetic clocks or epigenetic drift.

There has been considerable interest and excitement in the use of epigenetic age as a measure of biological age to predict the risk for aging-related diseases such as cancer. It is plausible that the stochastic process inherent in epigenetic drift can induce aberrant methylation events that accumulate in normal cells and eventually induce cancer formation, as suggested by a common set of changes in the DNAm pattern during aging and in cancer (22). This process is akin to the role of random mutagenesis in age-related cancer. For those clones that acquire a cancer-promoting epigenetic alteration secondary to epigenetic drift or clock phenomenon, they outcompete other clones and contribute to the clonal evolution of the cancer. The concept that age-dependent DNAm could predispose normal cells to cancer is supported by the observations of abnormal epigenetic-drift–related changes in various normal tissues at risk for cancer formation, such as the colon (54), the buccal mucosa of smokers (72), the hematopoietic stem cells of the elderly (73), and the skin (74). Furthermore, Klutstein and colleagues demonstrated that tissue-specific cancer risk is highly correlated with the degree of aberrant age-dependent methylation in normal tissues (75). Altogether, considerable evidence suggests that age-associated epigenetic drift or epigenetic aging may predispose normal cells to transforming into cancer cells.

Recently, studies have found epigenetic age acceleration in the blood samples collected from patients who developed cancer at follow up, compared with cancer-free participants (33, 76–78). It is noteworthy that the association of epigenetic age acceleration in the blood samples with cancer risk likely reflects a systemic effect of DNAm changes on cancer predisposition, such as through immune system alterations captured in the leukocyte DNAm alterations, which is in distinct contrast with epigenetic drift in the tissue samples, which may predispose to cancer via altering tumor suppressor genes and/or oncogenes that drive the expansion of cancer-initiating clones.

If epigenetic aging does affect cancer formation, how does it do this?

It is well accepted that the majority of human cancers develop via the accumulation of genetic and epigenetic alterations over time (79). However, the vast majority of aberrant methylation events in cancers, even when affecting gene promoters, do not appear to effect gene expression because the majority of genes that undergo aberrant DNAm are not expressed in normal adult tissues (80, 81). This observation raises interesting questions of how epigenetic aging contributes to cancer formation and what leads to certain gene-associated CpGs or CGIs being selected for methylation in cancers. One possibility is that a time-dependent stochastic event process, like epigenetic drift, could lead to cancer formation through the accumulation of random epigenetic alterations that, through chance, eventually alter epigenetic driver gene expression, leading to a clone of cells destined to become cancer. Indeed, although the phenomenon of (epi)genetic drift is generally associated with phenotypic neutrality, it is possible that coordinated or collective drift at the CGI level may cease to be neutral as methylation levels exceed certain thresholds that correlate with the potential to alter tumor suppressor genes or oncogenes by interfering with the transcriptional regulation at gene promoters, insulators, and enhancers (51, 82). One concrete example demonstrating how age-related DNAm could promote cancer formation is *HAND2*, a gene encoding a member of the basic helix–loop–helix family of transcription factors highly expressed in the endometrial stroma. The promoter of *HAND2* undergoes hypermethylation with age and during endometrial cancer development, leading to *HAND2* gene expression silencing (83). In fact, *HAND2* is found to be one of the most commonly silenced genes in endometrial cancer. Meanwhile, endometrium-specific knockout *Hand2* mice developed precancerous lesions with aging, providing further insight into our understanding of the functional role of epigenetic drift in cancer formation. In a study of DNAm and gene expression in BE and esophageal adenocarcinoma (42), we found significant negative correlations of gene expression

with advanced epigenetic drift in 87 TCGA esophageal adenocarcinoma samples. Among the ~200 gene–CGI pairs that exhibited significantly correlations between gene expression and methylation levels were several genes that are commonly methylated in BE or have been implicated in esophageal carcinogenesis (42, 84). In colorectal cancers, we similarly found that advanced epigenetic drift was frequently (in over 50% of drift-related CGI in the right colon) associated with significant reductions in gene expression. In contrast, we found only a small number of drift-related CGI–gene pairs for which drift correlated positively and significantly with gene expression. These were mostly at CGIs located in the gene body (41). Although our analysis does not demonstrate causality, the fact that epigenetic drift at CGIs in normal colon is more prominently associated with transcriptional changes in colorectal neoplasia than CGI that undergo little or no drift in normal colorectum suggests a potential role of epigenetic drift in the molecular pathogenesis of cancer. Of particular interest, epigenetic aging may target and silence specific transcription factors that function as tumor suppressors. Indeed, a comprehensive analysis of transcription factor landscapes revealed that bivalently and PRC2-marked transcription factors highly expressed in normal tissue are prone to be inactivated in cancer, predominantly via promoter hypermethylation (85).

Can epigenetic aging be slowed or reversed, and would this alter the risk of cancer?

Provocative and unresolved issues related to epigenetic drift and cancer formation include whether environmental factors can affect the pace of this process; and what is the relative importance of age-related epigenetic alterations in comparison to gene mutations. The fact that the epigenetic state of the genome is plastic and modifiable, unlike the genetic state, suggests that the possibility of slowing or reversing this process is plausible. There is ample evidence demonstrating that factors such as genistein and folate can alter the methylation status of CpGs in mouse models as well as compelling studies in humans associating environmental exposure history with aberrant DNAm (70, 86). Given the recent demonstration of the age-related accumulation in normal tissue of pathogenic gene mutations that clearly inactivate tumor suppressor genes and activate oncogenes, the potential role of concurrent epigenetic alterations playing a key and perhaps cooperating role in inducing the tumor-promoting events becomes more compelling (87, 88). If this proves to be true, it will be imperative to identify the environmental and lifestyle factors that can alter the epigenome to avoid or reverse disease-prone cellular states.

Closing Comments and Future Work

Aging, as a multifaceted and multidimensional process, is associated with increased risk for a number of diseases, including cancer. It is well appreciated that individuals age at different rates and that an individual's biological age and risk for age-related disease varies from his/her chronologic age. Furthermore, the risk for age-related diseases and cancer varies between individuals of the same chronologic age. It appears that some people exhibit age-acceleration and are at heightened risk for age-related diseases, whereas others exhibit age-deceleration and experience a healthier aging process (**Fig. 1**). Thus, improving our understanding of an individuals' biological aging process and the associated molecular alterations, as opposed to chronologic age, likely holds the key to improving our understanding of the age-related risk for diseases such as cancer. "Epigenetic drift," a stochastic process of global DNAm gain or loss as a function of age, emerges as a promising biomarker for biological aging and as a plausible molecular mechanism mediating this process. Out of thousands of CpG loci covering hundreds of CGIs that undergo drift or change significantly over time, various epigenetic clocks have been constructed to essentially assess different aspects of biological aging (**Fig. 2**). Among them, the Horvath and Hannum clocks may most closely reflect processes arising from an intrinsic aging process although they also appear to be affected by environmental exposures. Therefore, we propose that the Hannum and Horvath clocks may best be described as "hybrid chronological/biological clocks," a term brought up in a recent review article (89). In contrast, the epiTOC clock appears to measure the internal clock mechanism of stem cell divisions occurring during aging. Furthermore, the more recently derived DNAm PhenoAge clock goes beyond merely being a chronological/biological age estimator and performs as a biomarker for predicting mortality, health span, cardiovascular, or Alzheimer diseases (23).

Although much has been uncovered in linking epigenetic aging and cancer risk, several knowledge gaps need to be closed in the near future, particularly with regard to understanding the biological and clinical significance of the epigenetic drift phenomena. First, although previous studies have provided important information on the potential impact on gene expression imposed by the epigenetic drift, the functional consequences of epigenetic drift need to be further elucidated. Towards this end, integrative analysis of gene expression and epigenetic drift in both TCGA esophageal adenocarcinoma/colorectal cancer (EAC/CRC) dataset has uncovered a set of genes that appear to be subjected to epigenetic drift and transcriptional repression (40, 41). Future studies with emerging epigenomic editing tools are needed to characterize key drift CpGs or CGIs that may be causal in cancer initiation or progression, as it is likely that only a very small fraction of drift genes are of functional significance. Recently, aging-associated DNAm of multiple CGIs in normal tissue has been implicated in the origin of CIMP in colon cancer (90). It remains to be determined if individuals with accelerated aging rate might be at particular high risk for developing CIMP-high colorectal cancer, after normal colon cells have acquired spontaneous *BRAF* mutations. Second, it is plausible that individuals who have developed premalignant lesions and are at high risk of cancer exhibit age acceleration in their normal at risk tissue. Indeed, the epiTOC clock has demonstrated age acceleration in normal buccal tissue exposed to smoke and in normal breast tissue from patients with cancer. It is conceivable that an ideal "cancer risk clock" would perform in a tissue-specific manner, reflecting the unique intrinsic and extrinsic factors acting on the tissue under study. It is also important to keep in mind that the nexus between epigenetic aging and cancer risk could be more complicated than we expect it to be. Thus, to further advance our understanding of epigenetic aging and cancer, we will need large collections of normal tissue samples that have been characterized for their methylomic changes and that have rigorous risk factor annotation.

In summary, despite recent gains in our knowledge of aging and epigenomics, we still have an incomplete understanding of how aging, the major risk factor of human cancer, increases cancer risk. Undoubtedly, epigenetic aging is not simply a "molecular clock" when it comes to cancer. Opening up the epigenetic clockwork and seeing how it unwinds as we age may help us to better understand how normal tissues evolve, over time, into cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Yu, W.D. Hazelton, G.E. Luebeck, W.M. Grady
Development of methodology: W.D. Hazelton

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): W.D. Hazelton
Writing, review, and/or revision of the manuscript: M. Yu, W.D. Hazelton, G.E. Luebeck, W.M. Grady

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