HYPOTHESIS

Epigenetic clocks, aging, and cancer

Global methylation changes in aging cells affect cancer risk and tissue homeostasis

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Cancer and aging are accompanied by stereotyped changes to the epigenetic landscape, including progressive loss of DNA methylation over gene-poor genomic regions (*1*, *2*). Global hypomethylation arises in cells that have undergone many divisions, likely owing to imperfect maintenance. Evidence suggests that global hypomethylation represents a "mitotic clock" that counts divisions in somatic cells and functions to restrain aging cells and limit malignant progression. Therapies that modulate the pace of methylation loss or eliminate hypomethylated cells could alleviate aging-associated diseases or cancers.

Several lines of evidence indicate that hypomethylation represents a general mitotic clock (*3*, *4*). In tumors, hypomethylation affects megabase-sized intervals that are CpG-poor, gene-poor, and late-replicating. Tissues from older individuals also exhibit widespread hypomethylation of the same regions as in cancer, albeit of lesser magnitude. Hypomethylation is more pronounced in proliferative epithelial tissues, compared with brain tissue and other compartments that largely comprise postmitotic cells. Moreover, primary cells become progressively hypomethylated with increasing passages in culture, but their methylation stabilizes when they exit the cell cycle (*5*). In cancer cells, the degree of hypomethylation correlates with somatic mutational burden, which is established to have mitotic clock–like properties because the number of mutations increases as cells accumulate divisions (*6*).

DNA methylation loss associated with cell divisions is likely a consequence of inefficient epigenetic maintenance (see the figure). Methylation is copied during DNA replication by DNA methyltransferases (DNMTs). However, maintenance is less efficient in CpG-poor regions that replicate late in S phase, particularly in rapidly dividing cells. A study of replicating embryonic stem and HeLa cells showed that although methylation was copied to daughter DNA strands, it was delayed and error prone in late-replicating regions (*7*). Thus, global hypomethylation is a shared feature of aging and malignant cells rooted in inefficient maintenance and gradual methylation loss as somatic cells accumulate divisions.

The mitotic hypomethylation clock is distinct from clocks that predict chronological age from methylation in tissues. These organismal aging clocks have attracted considerable attention for their accuracy and potential to predict morbidity and mortality (*8*). Machine-learning algorithms identify and integrate sets of CpGs whose methylation status correlates with age. Although aging clocks have been developed for a range of tissues and organisms, different algorithms for the same tissue type incorporate different CpGs. This inconsistency has hindered interpretation of the methylation changes.

Organismal aging clocks presumably incorporate multiple inputs, including cell type composition, environmental influences, mitotic clock features, and other disruptions that arise over time. The mitotic clock input likely includes global hypomethylation as well as focal hypermethylation of specific CpG islands, which can also accumulate as cells divide. Global hypomethylation and focal hypermethylation both loosely correlate with chronological age (*3*, *9*). A relationship between mitotic and aging clocks is also supported by the observation that telomerase reverse transcriptase (TERT) variants that increase telomere length are associated with increased epigenetic age predictions, on the basis of measurements of DNA methylation in human peripheral blood mononuclear cells (*8*). Because these variants allow cells to undergo more divisions before telomere crisis (when telomeres become too short and cells arrest), a potential explanation is that more division increases mitotic methylation changes, which accelerates the aging clock.

The relationship between mitotic and aging-associated methylation changes is confounded by cell type heterogeneity. The pace of mitotic hypomethylation varies across cell types, with embryonic stem cells representing an extreme because they remain stably methylated (*3*, *10*). Epigenetic age also varies between individual cells in a tissue, proceeding slowly in certain progenitor populations (*11*). Although the precise relationship between mitotic and aging clocks remains obscure, the cell-intrinsic properties and mechanistic underpinnings of the DNA hypomethylation clock provide a complementary framework for the study of human biology and aging.

What is the impact of hypomethylation? Its prominence in cancer prompted extensive exploration of potential oncogenic functions, yet decades of study have failed to clarify such roles. Hypomethylation in tumor cells may alternatively represent a scar of having undergone many divisions rather than being a driving feature of malignancy. The recognition that hypomethylation is a consequence of accumulated divisions suggests that it might instead prune aging cells, protecting them from malignant progression and affecting aging tissue homeostasis.

Substantial evidence supports several such restrictive functions for hypomethylation. DNA methylation plays a fundamental role in the transcriptional silencing of transposable elements (TEs) such as endogenous retroviruses (ERVs) and long interspersed nuclear elements (LINEs), which are inserted at high copy numbers throughout mammalian genomes (*12*). The expression of TEs is strongly up-regulated across a range of tumors, particularly in hypomethylated regions. In support of a causal role for methylation loss, the DNMT inhibitor 5-azacytidine induces LINE and ERV expression in vitro and in vivo (*13*).

TE reactivation triggers innate immunity through viral mimicry (*13*). The derepressed elements produce double-stranded RNAs, which are sensed by cytosolic sensors that trigger a type I interferon (IFN) response. DNMT inhibitors activate TEs in cancer cells, increase immune infiltration, and sensitize tumors to immunotherapy. LINE reactivation has also been documented in senescent cells, where it triggers IFN responses and may promote inflammaging, a chronic inflammatory state linked to aging-associated diseases (*12*).

Global hypomethylation also alters chromatin topology, which can affect the phenotypes of neoplastic and aging cells. Late-replicating genomic regions localize to the nuclear periphery and lamina in healthy cells but relocate to the interior and gain repressive histone modifications in hypomethylated tumor cells (*5*, *14*). These architectural changes are accompanied by transcriptional

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changes likely to affect tumor and aging phenotypes. In contrast to TEs, the expression of most protein-coding genes in the reorganized regions is down-regulated in association with DNA methylation loss and chromatin changes. The deregulated genes are enriched for functions related to stem cell proliferation, epithelial-mesenchymal transition, and invasion (*14*). Their repression may restrain the proliferative and invasive potential of aging cells and hinder malignant progression.

Additional evidence suggests links between global hypomethylation and senescence, a conserved program that limits the proliferation of damaged cells (*5*). Senescent cells exhibit global hypomethylation and profound chromatin architecture changes, including the formation of senescence-associated heterochromatin foci (SAHFs). Key features of SAHFs, including their spatial redistribution and their association with repressive histone modifications, are reminiscent of reorganized chromatin in hypomethylated aging and malignant cells. These parallels raise the possibility that hypomethylation and associated chromatin changes also promote senescence.

DNA methylation is also critical for nuclear and genome integrity. Global hypomethylation could contribute to tumor initiation and evolution by promoting chromosomal instability (*4*). At the same time, p53-dependent DNA damage responses associated with hypomethylation consequent to DNMT inhibition or haploinsufficiency may cause cell cycle arrest or apoptosis. Aging cells also harbor cytoplasmic chromatin fragments enriched for markers of DNA damage and heterochromatin that are capable of activating the inflammatory stimulator of interferon genes (STING) pathway (*12*). Hence, although hypomethylation may contribute to the evolution of tumors, its net impact is more likely to restrain the proliferative capacity and plasticity of aging cells and to promote their immune clearance. In addition to checking malignant progression, effects of these sequelae on tissue homeostasis may confer other adaptive or detrimental aging phenotypes.

To the extent that global hypomethylation enacts barriers to proliferation and malignant progression, tumor cells must adopt strategies to overcome them. Certain tumors arise from stem cells unaffected by hypomethylation, whereas others acquire hypermethylating phenotypes that may slow or reverse mitotic hypomethylation (*3*). Activation of TEs and downstream sensing pathways may be muted by chromatin regulators or immune factors, which are frequently altered in cancer. Cancer cells may also benefit from the derepression of certain TEs, such as the ERVs that activate oncogenes in colorectal tumors (*12*). These competing forces allude to a battle between organismal evolution and tumor evolution, with the former yielding tumor-suppressive features of the hypomethylation clock and the latter enabling malignant cells to overcome or exploit those features.

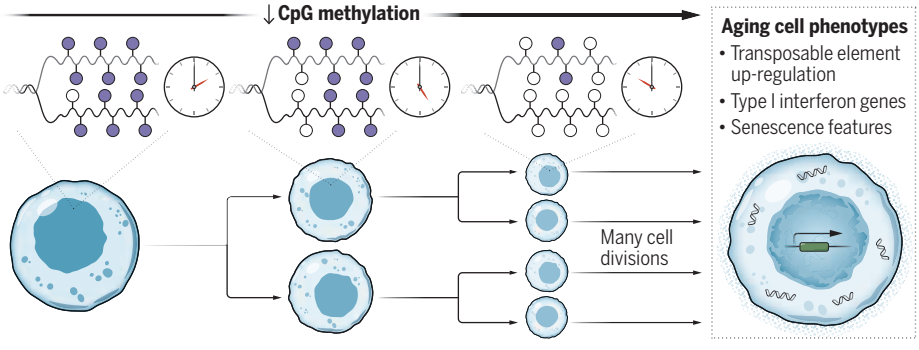
Although hypomethylation is conserved across mammals, its pace varies between organisms and cell types. Accelerated global hypomethylation was proposed to explain the rarity of tumors in blind mole rats, a species notable for longevity and cancer resistance (*15*). So although hypomethylation may reflect an inherent feature of mammalian genomes, it may be "tunable" across species and tissues. This is reminiscent of the heterochromatin-based mitotic clock in the yeast *Saccharomyces cerevisiae*, whose modulation alters life span (*5*). There are also parallels to somatic mutational rates, whose variability across mammals scales with life span (*6*). Moreover, the extent to which hypomethylation primes downstream expression changes and phenotypes may represent an additional tunable component of the mitotic clock. Even to the extent that hypomethylation is an intrinsic feature of mammalian genomes predicated in imperfect maintenance, its pace and downstream integration by chromatin and transcriptional machinery have likely evolved to balance cancer resistance and aging phenotypes.

It should be recognized that this model of mitotic hypomethylation and its impact on cell phenotypes remains nascent and largely inferential. The hypomethylation landscape has been defined from profiles of tumors, heterogeneous tissues, and cultured cell lines, but the patterns and pace of methylation loss will vary according to cell lineage, developmental stage, and environment. New tools are needed to resolve methylation patterns across single cells in heterogeneous tissues and to track methylation loss in real time. Furthermore, downstream functional consequences have been largely inferred from correlations with chromatin states and TE expression. They are supported by genetics and the effects of DNMT inhibitors, but these do not fully recapitulate the hypomethylation in aging and malignant cells. Other key questions relate to the ubiquity of hypomethylation in tumors and how cancer cells overcome the associated barriers.

A more detailed understanding of the hypomethylation clock could reveal biomarkers that predict disease risk or stratify tumors. It could yield new therapeutic modalities that prune aging or premalignant cells by exploiting vulnerabilities associated with hypomethylation. Alternatively, therapies that slow or reverse cell-intrinsic DNA hypomethylation might alleviate inflammation or other aging-associated deficits. Interventions will need to strike a balance to improve aging tissue homeostasis without potentiating cancer risk. ■

The mitotic hypomethylation clock restrains aging cells

DNA hypomethylation arises from inefficient maintenance of CpG methylation in late-replicating genomic regions with low CpG density. The baseline methylation of these regions (purple circles) can gradually be lost (empty circles) as cells divide. Eventually, global hypomethylation may arise, which restrains aging cells and hinders tumor progression through the induction of transposable element expression, innate immunity (interferon responses), and senescence-associated chromatin alterations.



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