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Special Article

Epigenetic aging of colorectal mucosa in cancer development

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Abstract

Background: The past decade has seen the development of epigenetic models of aging that accurately estimate chronological age and predict disease incidence and mortality. These estimates are modulated by lifestyle and environmental factors linked to carcinogenesis, but to date this has primarily been studied in blood.

Methods: We examined epigenetic aging in normal colonic tissue (n = 96), adjacent mucosa (n = 245), and tumors (n = 208), using models trained on age (Horvath, Hannum, Zhang), mortality (PhenoAge, GrimAge), aging rate (DunedinPACE), cellular mitotic history (EpiTOC, epiTOC2, miAGe), and telomere length (DNAmTL).

Results: The Horvath model was the most accurate estimator of chronological age in normal colonic mucosa, with high correlation ($r > 0.70$) between the Horvath, Hannum, Zhang, PhenoAge, and GrimAge models as well as between mitotic clocks ($r > 0.94$). All models showed similar performance in normal tissue and adjacent mucosa but substantially more variation in estimates in tumors. Statistically significant differences in age acceleration were present between normal and adjacent mucosa by 6 models (Hannum, Zhang, PhenoAge, EpiTOC, epiTOC2, and miAge), while tumors showed highly statistically significant differences by all models. Age acceleration differed by region of the colon, with varying patterns by model type. Physical activity (PhenoAge), smoking history (GrimAge), and alcohol consumption (Horvath, mitotic clocks) were associated with epigenetic aging in adjacent mucosa, while smoking history, smoking intensity, and alcohol consumption were associated with DNAmTL in tumors.

Conclusions: Our study reveals an impact of tissue type, region, and lifestyle factors on epigenetic aging and highlights heterogeneity between models and the need for careful consideration within study design.

Introduction

The development of colorectal cancer (CRC) is driven by a range of genetic and epigenetic factors. Epigenetic changes occur early in carcinogenesis[1] and are observable in the mucosa adjacent to tumors[2,3] and in adenomas.[4] There is notable overlap between the epigenetic changes observed in colorectal tumors and those associated with aging in the colonic mucosa.[5]

The past decade has seen the development of a range of epigenetic models of aging that accurately estimate chronological age and predict disease incidence and mortality. The earliest

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"epigenetic clocks," by Horvath[6] and Hannum et al.,[7] estimate chronological age to within 6 years. Further models have been developed with increased accuracy in estimating chronological age (eg, Zhang et al.[8]) or trained to predict mortality (PhenoAge[9] and GrimAge[10]) or aging rate (DunedinPACE[11]). Other models use epigenetic profiling to report on cellular traits, such as cumulative mitotic history (EpiTOC,[12] epiTOC2,[13] and miAGE[14]) and telomere length (DNAmTL[15]). Subsequently, there are now a plethora of epigenetic aging models, variously trained on chronological age, mortality, mitotic history, and lifestyle factors.[16] Although some were developed for specific tissues,[17,18] they have predominantly been trained using whole blood samples.

Epigenetic aging in blood is predictive of disease incidence and outcomes, including incidence of breast[19] and lung cancers[20] and all-cause mortality in the elderly.[21] It is modulated by lifestyle and environmental factors linked to carcinogenesis, including smoking, alcohol, body mass index (BMI), and diet.[22-25] However, such studies have near exclusively been performed in blood, with little examination of other tissues and no exploration of how lifestyle factors influence epigenetic aging in the colorectum. Although epigenetic aging can be systemic (and thereby captured by blood-based analysis), it can be tissue-specific or even discordant between tissues as the product of differential impact of environmental factors and intrinsic differences in rates of cellular proliferation and senescence.[26] Models trained on blood have nonetheless been used in a limited number of studies of breast[27] and colorectal[28,29] tissues, but their performance in nonblood tissues has not been evaluated.

We investigated epigenetic aging in normal colonic mucosa and adjacent mucosa and tumors from CRC patients. We evaluated 10 models trained on different criteria and explored differences in epigenetic aging by model, tissue type, and region of the colorectum and in relation to lifestyle factors associated with CRC incidence.

Methods

ColoCare samples and supporting datasets

Samples of adjacent mucosa (n = 149) and tumors (n = 112) from patients within the ColoCare Study,[30] including 104 matched pairs, were analyzed by Illumina Infinium HumanMethylation450 BeadChip microarray. Publicly available datasets were used to support and enhance analysis of the ColoCare dataset. Samples of normal mucosa (n = 48), adjacent mucosa (n = 96), and tumors (n = 96) were obtained from Díez-Villanueva et al.[31] (Colonomics; GEO accession number: GSE131013), and normal mucosa (n = 48; low-risk samples with no concurrent adenomas) from Wang et al.[32] (GSE132804).

Details of the ColoCare Study and lifestyle covariates are outlined within the Supplementary Methods. Patient characteristics are provided within Tables S1 and S2.

Calculation of epigenetic aging

Microarray datasets were normalized together using the R minfi package and Regression on Correlated Probes (RCP), and all analyses performed together unless otherwise stated (Figure S1). A total of 379 895 loci passed quality control and filtering stages. Epigenetic aging estimates for the Horvath, Hannum, Zhang, PhenoAge, GrimAge, DunedinPACE, EpiTOC, epiTOC2, and DNAmTL models were calculated using the R package DNAMethyAge,[33] and miAge using methylCIPHER.[34] Of targeted probes, 91%-100% were available within the normalized dataset (Table 1), with missing values imputed.

Statistical analysis

Pearson correlation coefficients were calculated for correlations between chronological age and epigenetic age and between the outputs of different models. Age acceleration, calculated as epigenetic age regressed on chronological age, was used for comparison of tissue types (normal mucosa, adjacent mucosa, and tumors) and regions (proximal, and distal or rectal). Differences were determined by Mann–Whitney U test, with no adjustment for paired adjacent mucosa and tumor samples from patients, consistent with previously published epigenetic analyses of paired tissues.[35–38] Correlations between epigenetic age estimates in paired samples of adjacent mucosa and tumors were calculated by Spearman rank correlation test. Associations between epigenetic age acceleration and lifestyle factors were determined by linear regression, adjusted for tumor site. Where relevant, *P* values were adjusted for multiple testing using the Benjamini–Hochberg procedure for false discovery rate (FDR), with statistical significance defined as a *P* value less than .05. All analyses were conducted in R (v4.5.0).

Results

We selected 10 models based on their use in previous studies of cancer risk and frequency of use across all studies of epigenetic aging and to reflect the range of models trained on different cellular and aging processes. We categorized the models as those trained on chronological age (Age Clocks; Horvath, Hannum and Zhang), mitotic history (Mitotic Clocks; EpiTOC, epiTOC2, and miAGe), mortality (Mortality Clocks; PhenoAge and GrimAge), rate of aging (DunedinPACE), and telomere length (Telomere Clock; DNAmTL) (Table 1). We applied these models to samples of normal colonic mucosa (n = 96), tumors (n = 208), and adjacent

Table 1. Overview of the assessed epigenetic aging models used in the study.[a]

Model	CpGs, No.	Available, No. (%)	Category	Training tissue	Output	Development
Horvath	353	347 (98)	Age	51 tissues	Epigenetic age	Horvath[6]
Hannum	71	70 (99)	Age	Blood	Epigenetic age	Hannum et al.[7]
Zhang	514	488 (95)	Age	Blood, saliva	Epigenetic age	Zhang et al.[8]
PhenoAge	513	497 (97)	Mortality	Blood	Epigenetic age	Levine et al.[9]
GrimAge	1030	958 (93)	Mortality	Blood	Epigenetic age	Lu et al.[10]
DunedinPACE	173	157 (91)	Rate of aging	Blood	Rate of aging	Belsky et al.[11]
EpiTOC	385	385 (100)	Mitotic	Blood	Cumulative mitotic history	Yang et al.[12]
epiTOC2	163	163 (100)	Mitotic	Blood	Cumulative mitotic history	Teschendorff[13]
miAge	286	266 (99)	Mitotic	8 tumor types	Cumulative mitotic history	Youn and Wang[14]
DNAmTL	140	131 (94)	Telomere length	Blood	Telomere length estimate	Lu et al.[15]

[a]Details are provided regarding number of CpG sites used by each model, the tissue type used in the original development of each model, and the form of output.

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histologically normal mucosa (n = 245), with the total dataset analyzed together unless otherwise stated (Figure S1).

Correlation of epigenetic age estimates with chronological age in colonic tissues

Epigenetic models of aging have primarily been trained using blood samples. To characterize their accuracy in colonic mucosa, we applied the same 3 statistical measures as Horvath[6]: Pearson correlation between chronological age and epigenetic age; median absolute difference between these values; and mean age acceleration. Five models evaluated here produce age-like outputs (ie, numerical values resembling chronological age): the Age and Mortality Clocks. Of these, the Horvath model demonstrated the highest accuracy in normal colonic mucosa (Figure 1). GrimAge showed the highest correlation with chronological age ($r = 0.97$), while outputs were systematically higher, potentially reflecting calibration bias from applying a blood-trained clock to nonblood tissue. In contrast, the pan-tissue Horvath model showed a high correlation ($r = 0.86$) and low median absolute difference to chronological age (median absolute difference = 4.0) (Table S3). The Hannum, Zhang, and PhenoAge models showed moderate correlations ($r = 0.65$-0.76) and accelerated age estimates (mean age acceleration = 22.3, 19.1, and 7.2 years respectively).

The 5 models showed comparable performance in adjacent and normal mucosa, with the Horvath Clock consistently demonstrating superior accuracy across both tissue types. Although correlations with chronological age were only marginally lower in adjacent mucosa, there was increased variance in epigenetic age estimates (Figure 1). In tumors, correlations with chronological age showed a marked decline for the Horvath, Hannum, Zhang, and PhenoAge models ($r < 0.44$) and median absolute differences increased to more than 10.5 years. Uniquely, the GrimAge model retained highly comparable performance across all tissues.

Performance of mitotic and other clocks in colonic tissues

The Mitotic Clocks showed a near identical performance to one another (Figure 2). Their correlations with age were of similar magnitude in normal mucosa ($r = 0.39$-0.45), adjacent mucosa ($r = 0.43$-0.50), and tumors ($r = 0.11$-0.22). They did not show increased variability in adjacent mucosa in comparison with normal tissues but still displayed weaker correlations with chronological age and increased variance in tumors.

DNAmTL showed an inverse correlation with chronological age, of similar magnitude to Mitotic Clocks ($r = -0.44$ to -0.41 and $r = -0.14$ in normal, adjacent, and tumor tissues, respectively), with no difference in variance between normal and adjacent mucosa. The DunedinPACE model was unique in showing no correlation with chronological age in normal tissue ($r = 0.15$), adjacent mucosa, ($r = -0.06$), or tumors ($r = 0.06$).

Correlation of epigenetic aging estimates between models

We next examined correlations between the outputs of the 10 models. In normal mucosa, this revealed 2 distinct subgroups (Figure 3): one comprised of the Horvath, Hannum, Zhang, PhenoAge, and GrimAge models, with correlations of 0.70-0.92 between their outputs; the other comprised of the Mitotic Clocks, with very high correlation coefficients ($r = 0.94$-1.00). The Hannum model was unique in having strong correlations with models within both groups. DNAmTL showed moderate-to-strong inverse correlations with the first subgroup, but

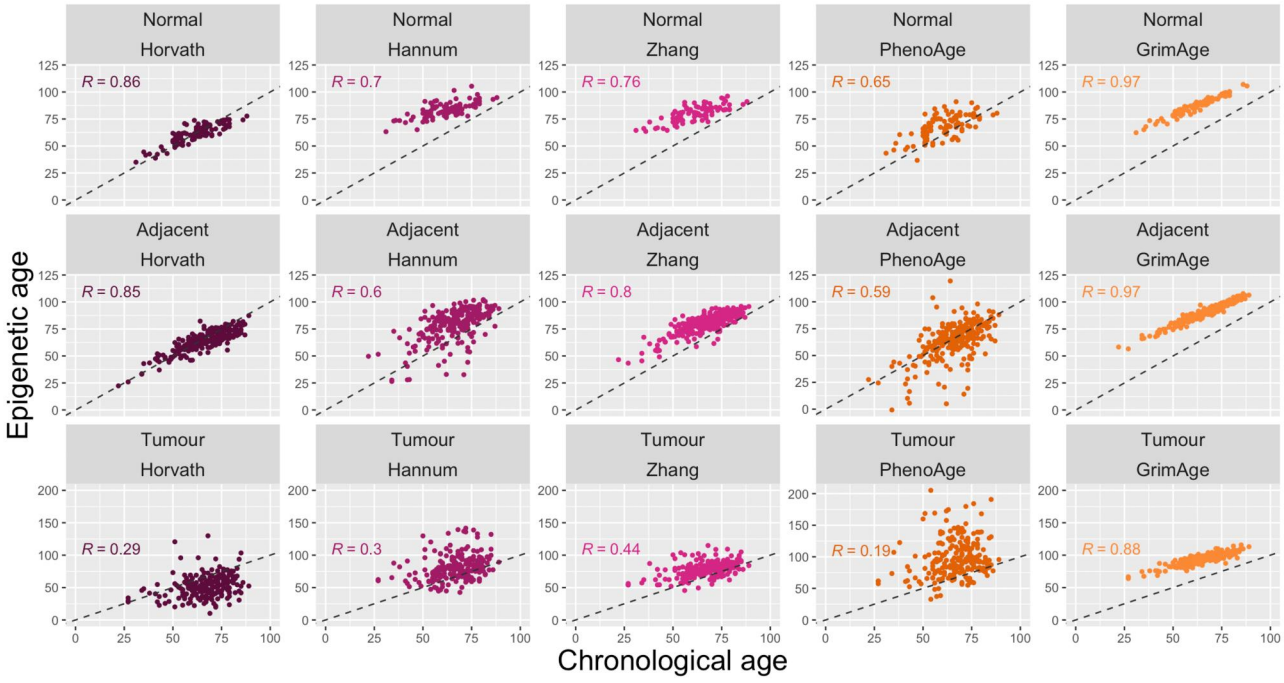

Figure 1. Performance of Epigenetic Clocks in colonic tissues. Correlation between chronological age and epigenetic age as calculated by the Horvath, Hannum, Zhang, PhenoAge, and GrimAge models, in samples of normal colonic mucosa from healthy individuals (top row), adjacent mucosa (middle row), and tumors (bottom row). Pearson correlation coefficients are indicated. The **dashed diagonal line** indicates perfect correlation between chronological age and epigenetic age.

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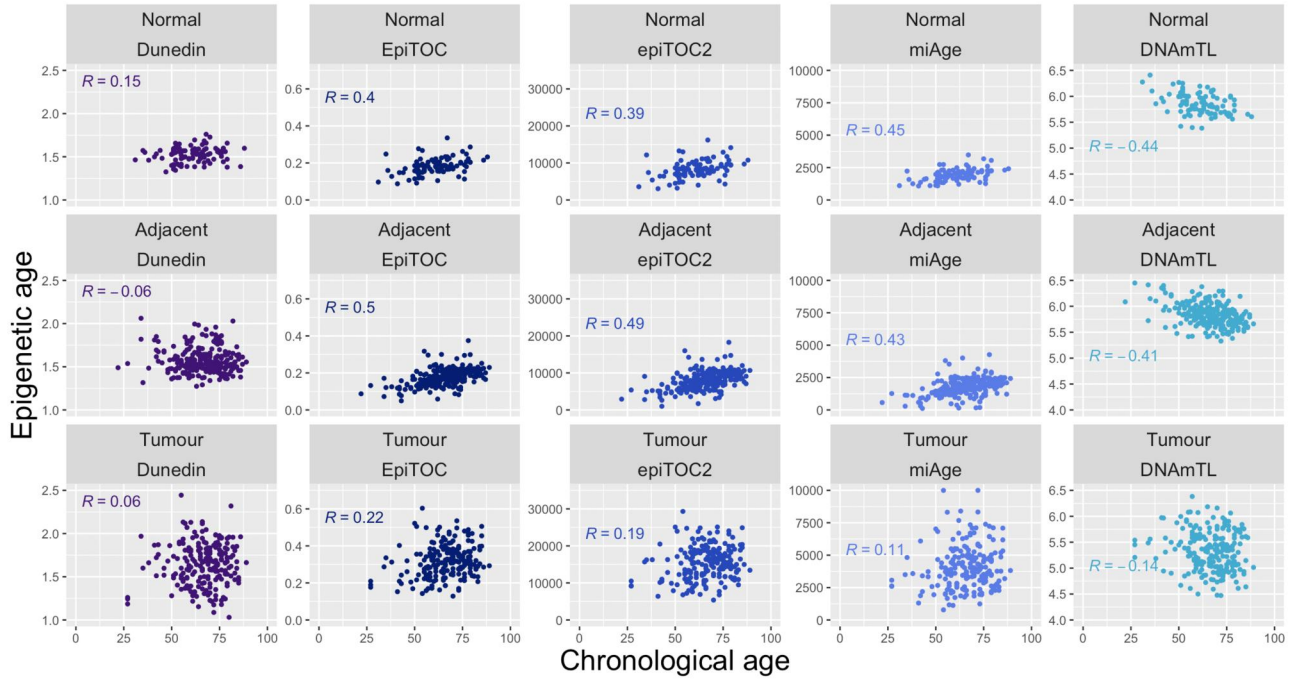

Figure 2. Performance of Mitotic and other clocks in colonic tissues. Correlation between chronological age and outputs generated by the DunedinPACE, EpiTOC, epiTOC2, miAge, and DNAmTL models, in samples of normal colonic mucosa from healthy individuals (top row), adjacent mucosa (middle row), and tumors (bottom row). Pearson correlation coefficients are indicated.

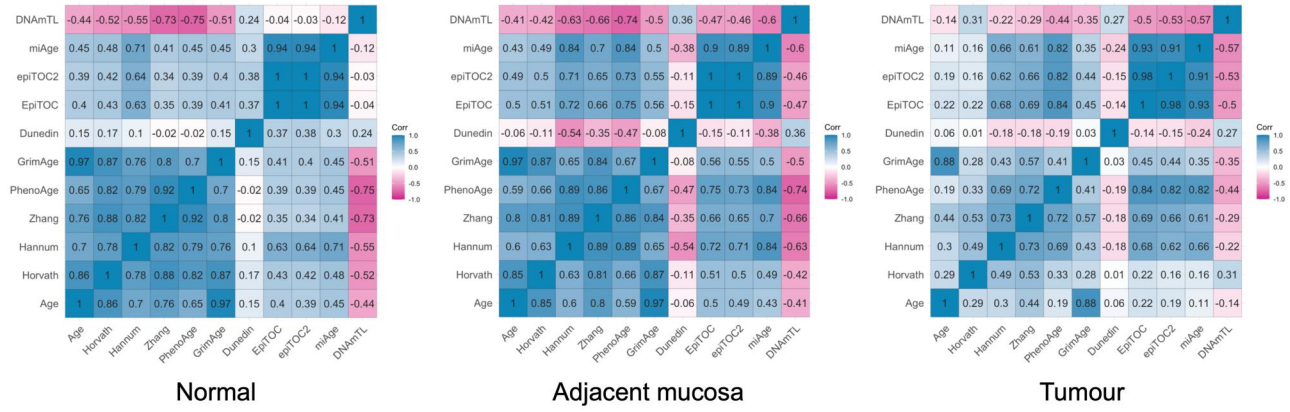

Figure 3. Correlation between epigenetic aging estimates by model. Pearson correlation coefficients between the outputs of 10 epigenetic aging models and chronological age in normal colonic mucosa from healthy individuals, adjacent mucosa, and tumors. Positive correlations are indicated in **blue**, and negative correlations in **pink**. corr = correlation.

unexpectedly no correlation with the Mitotic Clocks. DunedinPACE showed no strong correlations with any other model.

These subgroupings were largely retained in adjacent mucosa, with similar levels of correlation as in normal mucosa. In tumors, the models near universally showed weaker correlations. Interestingly, however, the Zhang, PhenoAge, and DNAmTL models displayed a pattern of decreasing correlation with Age Clocks and increasing correlation with Mitotic Clocks from normal mucosa to adjacent and tumor tissues (Figure S2).

Epigenetic aging of tumors and adjacent mucosa

We next applied the 10 models to investigate epigenetic aging in relation to colorectal carcinogenesis. Age Clocks displayed a pattern of statistically significant deceleration in epigenetic aging from normal to adjacent mucosa by the Hannum (Mann–Whitney U test: $P < .0001$) and Zhang ($P = .0001$) models and greater deceleration between normal and tumor tissue by all 3 models (Figure 4, A; Figure S3). Conversely, the Mortality Clocks showed highly statistically significant increases in epigenetic aging in tumors (both $P < .0001$), while adjacent mucosa showed a statistically significant decrease by the PhenoAge model ($P < .0001$) but not GrimAge ($P = .84$). DunedinPACE similarly showed an increase in rate of aging in tumors ($P < .0001$) but not adjacent mucosa ($P = .09$). Mitotic Clocks presented the strongest and most uniform pattern, with all 3 models displaying statistically significant decreases in adjacent mucosa compared with normal tissue (all $P < .0003$) and very highly statistically significant increases in tumors (all $P < 1 \times 10^{-29}$). DNAmTL showed an inverse pattern to the Mitotic Clocks (ie, higher values in adjacent

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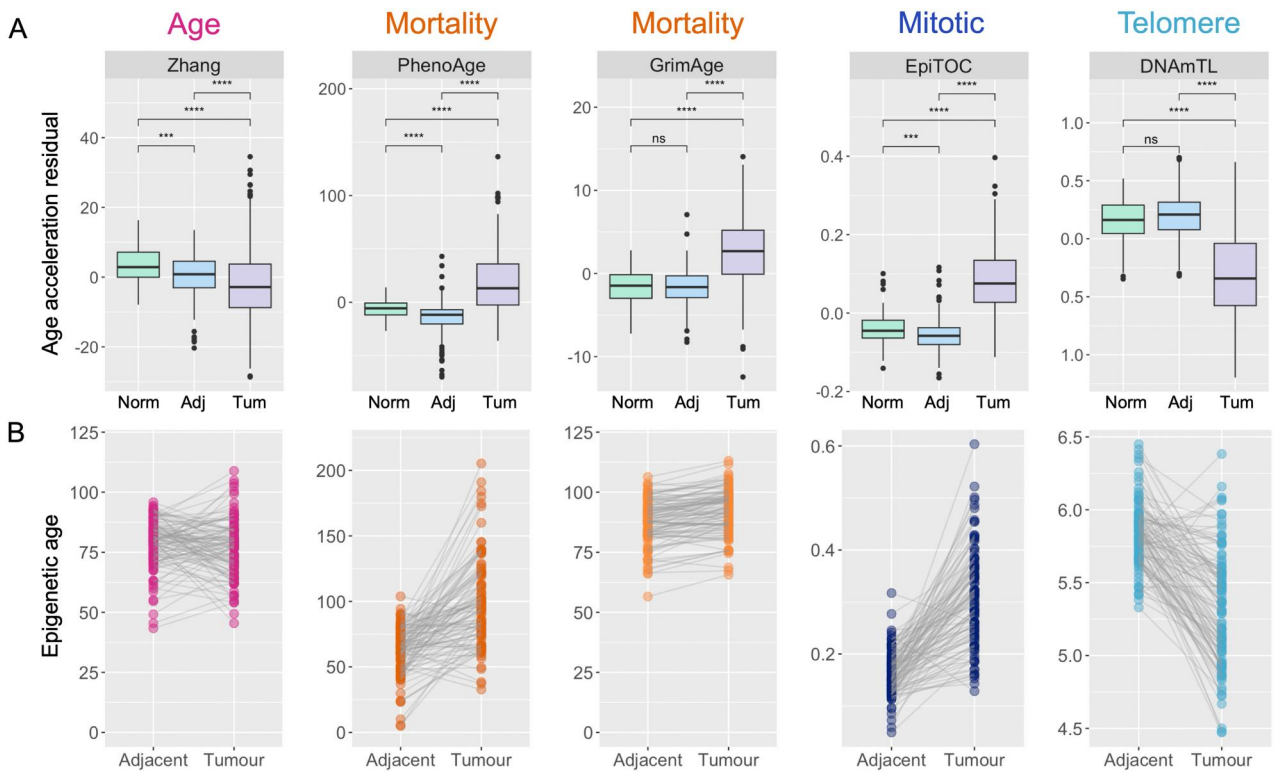

Figure 4. Epigenetic aging in tumors and adjacent mucosa. **A)** Age acceleration residuals in normal colonic tissue, adjacent mucosa, and tumors taken from all datasets generated by the Zhang (Age), PhenoAge (Mortality), GrimAge (Mortality), EpiTOC (Mitotic), and DNAmTL (Telomere) models. Statistical significance is indicated (Mann–Whitney U test; *$P < .05$; **$P < .01$; ***$P < .005$; ****$P < .001$). **B)** Epigenetic age in paired adjacent mucosa and tumor samples from the ColoCare study, as assessed by the Horvath (Age), PhenoAge (Mortality), GrimAge (Mortality), EpiTOC (Mitotic), and DNAmTL (Telomere) models. **Lines** indicate samples from the same patient. Adj = adjacent mucosa; Norm = normal; ns = nonsignificant; Tum = tumor.

mucosa and lower in tumors), but the difference between normal and adjacent mucosa did not reach statistical significance ($P = .17$). Cohort-specific analyses within samples from the ColoCare and Colonomics studies validated the statistically significant differences observed across the total dataset except for the Zhang model in normal vs adjacent mucosa ($P = .49$) and adjacent mucosa vs tumor ($P = .11$), and DunedinPACE for normal vs tumor ($P = .08$) and adjacent mucosa vs tumor ($P = .07$) (Tables S4-S6).

Comparison of matched tumor and adjacent mucosa pairs

We then compared epigenetic age estimates in paired samples of tumors and their adjacent mucosa in samples from the ColoCare Study (n = 104). Statistically significant differences in age acceleration residuals were present between the tissues for 8 of the 10 models. Tumors displayed epigenetic age acceleration by all 5 Mortality and Mitotic Clocks and by DunedinPACE ($P < .0001$) and deceleration by the Horvath and DNAmTL models ($P < 1 \times 10^{-8}$). However, we observed little correlation in epigenetic aging estimates between paired tissues from the same patient. GrimAge displayed a strong positive correlation (Spearman rank correlation: $r = 0.84$; Figure 4, B), but all other models displayed weak correlations ($r = 0.11$-0.29). In analysis by tumor location, GrimAge remained positively correlated in the proximal ($r = 0.92$, $P < .0001$) and distal colon ($r = 0.78$, $P < .0001$), while Dunedin PACE showed a moderate correlation specific to the distal colon ($r = 0.38$, $P = .02$).

Regional differences in epigenetic aging within the colorectum

Colorectal tumors exhibit heterogeneity in their molecular pathogenesis by their anatomical origin within the colorectum. We therefore examined differences in epigenetic aging by region (proximal vs distal and rectum). Normal mucosa in the distal colon displayed increased epigenetic aging by the Age Clocks and PhenoAge, lower mitotic turnover by the Mitotic Clocks, shorter telomere length by DNAmTL, and slower rate of aging by DunedinPACE (Figure 5). Adjacent mucosa similarly showed increased epigenetic aging in the distal colon by the Age Clocks, but no statistically significant differences by other models. Tumors in the distal colon consistently displayed lower turnover by the Mitotic Clocks and slower epigenetic aging by the Zhang and PhenoAge models.

The importance of regional differences within the colorectum is demonstrated by the statistically significant differences between the 3 tissues (normal, adjacent mucosa, and tumor) for some models being confined to either the proximal or distal regions. The Zhang model consistently showed significant differences between the 3 tissues in the distal colon (all $P < .0003$) but never the proximal (all $P > .93$; Figure S4). Although 7 models (Horvath, PhenoAge, GrimAge, EpiTOC, epiTOC2, miAge, and DNAmTL) displayed concordant and highly significant differences between tumors and both normal and adjacent mucosa irrespective of region, there was more variability when comparing normal tissue with adjacent mucosa. The Mitotic Clocks only displayed significant differences between normal and adjacent mucosa in the proximal colon (all $P < .005$) and never in the distal (all $P > .45$), while DNAmTL and PhenoAge showed the inverse (Figure S4).

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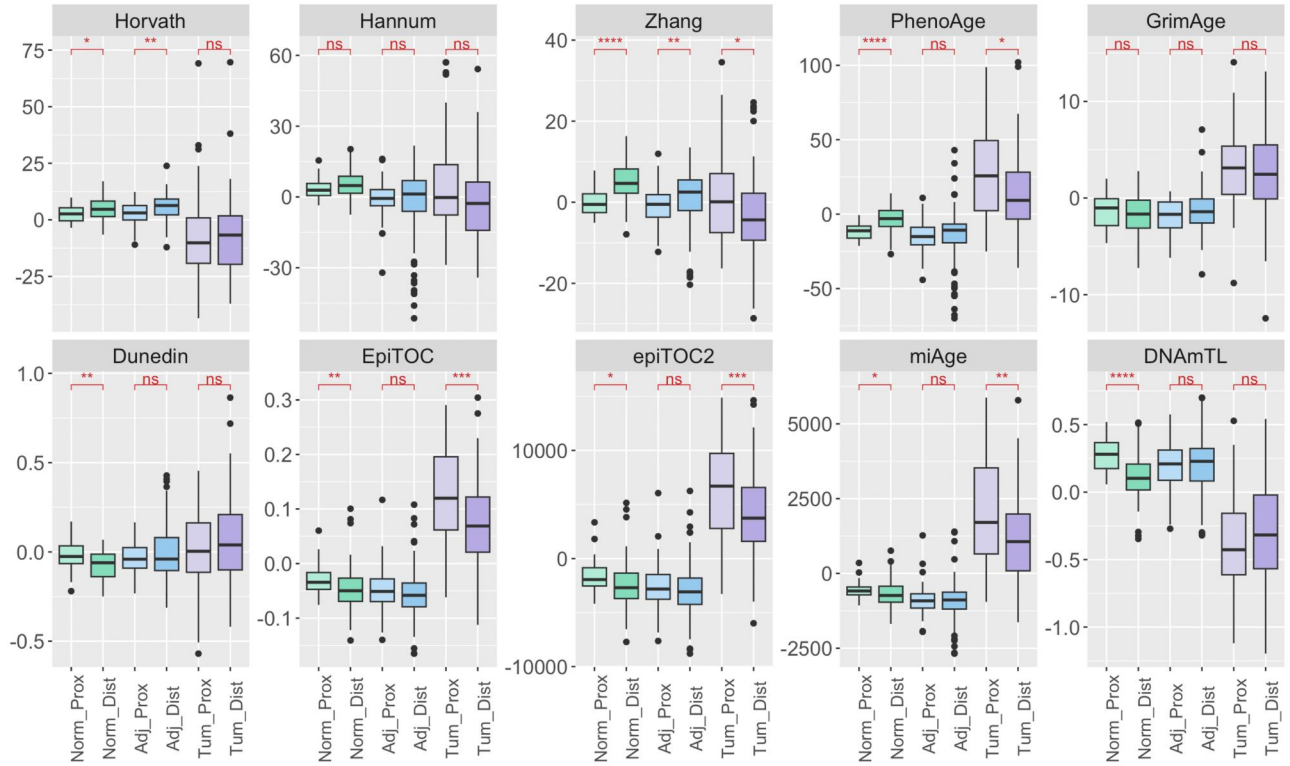

Figure 5. Epigenetic age estimates differ by region of the colorectum. Age acceleration residuals in samples of normal tissue (Norm; green), adjacent mucosa (Adj; blue) and tumors (Tum; purple) separated by region of the colon (proximal = Prox; distal = Dist). Statistical significance is indicated (ns = nonsignificant; $P > .05$; *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$).

Impact of lifestyle factors on epigenetic aging within the colorectum

We examined associations between alcohol consumption, BMI, smoking history, pack-years of smoking, and physical activity with epigenetic aging in adjacent mucosa and tumor tissue from ColoCare patients. In models adjusted for tumor location, physical activity was inversely associated with PhenoAge in adjacent mucosa ($P = 0.03$) and BMI with DunedinPACE in tumors ($P < .01$). Similarly, smoking was inversely associated with epigenetic aging in adjacent mucosa and tumors. Ever-smoking status was inversely associated with GrimAge in adjacent mucosa ($P = .02$), while DNAmTL was inversely associated with ever-smoking ($P = 0.03$) and pack-years of smoking ($P = .045$) in tumors. Alcohol consumption displayed mixed associations, trending toward accelerated aging in adjacent mucosa (Horvath, $P = .06$; EpiTOC, $P = .07$; epiTOC2, $P = .10$) and reduced telomere length in tumors ($P = .06$). Statistically significant observations are presented within Figure 6, and all results within Table S7.

Although multiple lifestyle factors were linked to epigenetic aging in the colorectum, these associations were moderate and did not remain statistically significant following correction for multiple testing. Sensitivity analyses revealed statistically significant associations between alcohol consumption and epigenetic aging models (Horvath, EpiTOC, and EpiTOC2 models in adjacent mucosa; DNAmTL in tumors) after removing outliers based on age at diagnosis (younger than 30 years).

Discussion

Epigenetic models of aging have gained prominent interest for their potential to reveal the impact of lifestyle and environmental factors in modulating disease risk. Despite their promise, questions remain about their applicability to different tissues and the inferences that can be drawn from the data. Our study has revealed divergent results between models, differences by region of the colorectum, and an impact of lifestyle.

As most studies of epigenetic aging and cancer risk have been performed using blood samples, a critical question is what tissue offers greatest predictive value. Moderate epigenetic age acceleration has been observed in the blood of CRC patients using the Horvath and Hannum models, [39,40] with conflicting findings for PhenoAge and GrimAge.[41,42] This age acceleration may be the product of accumulative lifetime exposures.[39] Examination of colorectal tissues could elucidate the impact of lifestyle factors directly on the tissue, but to date there have been few studies of this. Statistically significant deceleration of the PhenoAge clock has been reported in colonic mucosa from individuals with cancer,[32] while long-term aspirin usage slows epigenetic aging in the proximal colon by the Horvath, Hannum, and PhenoAge models[28] and Mitotic Clocks.[29] Our data demonstrate that epigenetic aging of the adjacent mucosa is more similar to normal mucosa than tumors but can still be discriminated from healthy mucosa taken from individuals free of cancer. However, it should be noted that one limitation of our study is the lack of normal tissue originating from the ColoCare Study, and although we have sought to minimize cohort-specific effects, we cannot exclude that this may have impacted the comparison of normal tissue to adjacent mucosa and tumors.

We observed heterogeneity in findings by model. The Mortality, Mitotic, and Telomere Clocks show strong discriminating power between nonmalignant mucosa and tumors, while the Age Clocks showed greater variability by model and region. The effectiveness of Mitotic Clocks is expected, because of their development to reflect mitotic turnover in relation to cancer

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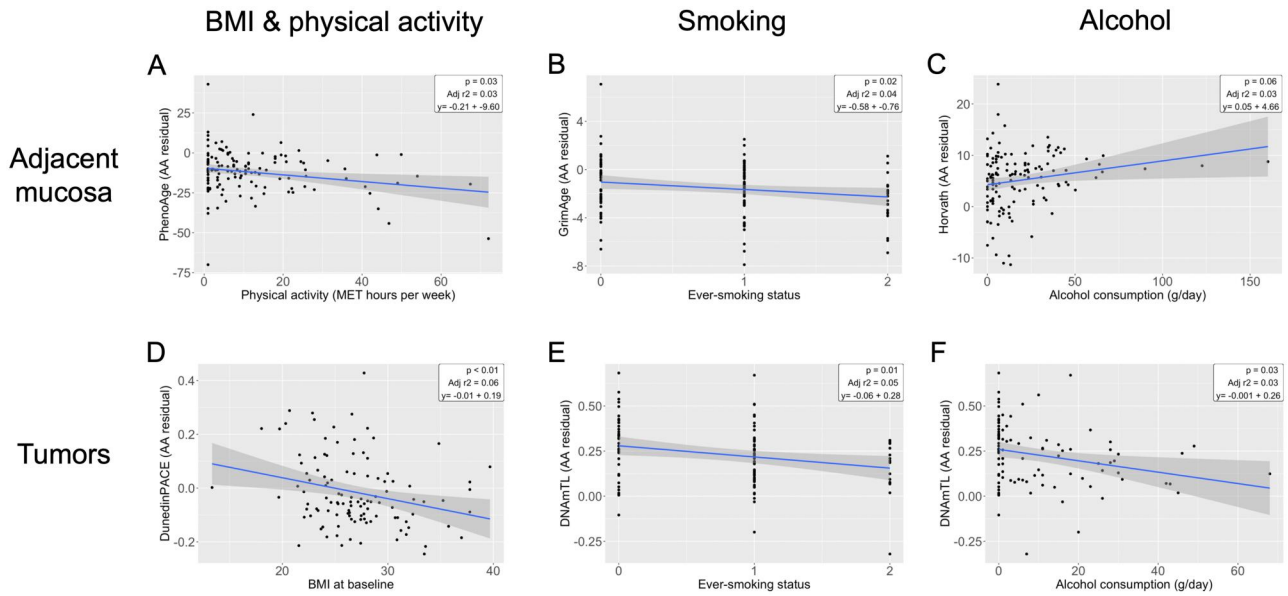

Figure 6. Impact of lifestyle on epigenetic aging in the colorectum. **A-C)** Associations between physical activity and PhenoAge **(A)**, ever-smoking and GrimAge **(B)**, and alcohol consumption and Horvath **(C)** in adjacent mucosa. **D-F)** Associations between body mass index (BMI) and DunedinPACE **(D)**, ever-smoking and DNAmTL **(E)**, and alcohol consumption and DNAmTL **(F)** in tumor tissue. Physical activity was recorded as metabolic equivalent (MET) hours per week, and BMI was assessed at baseline. Ever-smoking status was classified as never (0), former (1), or active (2) smoking at diagnosis. Alcohol consumption was recorded as average daily alcohol consumption (g/day) over the previous year. *P* values, correlation coefficients (adjusted r^2), and slopes are annotated. AA = age acceleration; Adj = adjacent mucosa.

risk.[12] However, our findings suggest there is weak correlation between epigenetic aging in tumors and adjacent mucosa. Therefore, analysis of histologically normal colonic mucosa may bring insight into carcinogenic processes; however, it is unlikely to be predictive of tumor properties. Of the Age Clocks, the Horvath model offers the highest accuracy, which is expected as it was developed as a pan-tissue clock[6] but showed no statistically significant difference between normal and adjacent mucosa. This demonstrates the value of tissue-trained or validated models but also the differential utility of models according to the biological processes they are trained on.

Our findings highlight the need to account for the anatomical location of the tumor. Our observations are supported by evidence elsewhere that the rate of age-related epigenetic drift varies by region of the colon.[43] While we, and others, have categorized samples (proximal, distal or rectal), the gradual differences in tumor properties across the colorectum suggest a continuum, rather than subdivisions.[44]

Epigenetic aging models can show tissue-specific effects in response to lifestyle and exposures.[25] We observed a small number of associations between lifestyle factors and epigenetic aging in colorectal tissues, with heterogeneity between models. The analogous relationships of BMI with epigenetic aging suggest that these factors may exert similar mechanisms of influence on the epigenome in colorectal tissues. This is in line with previous findings that excess adiposity is associated with accelerated epigenetic aging in blood.[23] The inverse association between smoking and epigenetic aging in adjacent mucosa is intriguing, as it contrasts with work elsewhere reporting accelerated epigenetic aging in the blood of smokers.[22] Similarly, the divergent patterns observed with alcohol consumption underscores the difficulty in interpreting these observations. It may reflect differential responses to alcohol exposure in normal vs tumor tissue, potentially through altered metabolic processes or gene expression patterns in tumors. However, although our study is the first to report an impact of lifestyle on epigenetic aging in the colorectum, these effects are comparatively moderate and come from different models, with no single model emerging as the most appropriate to examine the impact of lifestyle.

There are further lifestyle factors that we have not been able to assess here but that may impact epigenetic aging in this tissue. Diet modulates epigenetic aging in blood[24,45,46] but has not yet been examined in the colorectum. This may be a complex relationship mediated by food items such as red meat (eg, through increased inflammation[47]) and fiber (increased apoptosis and cellular turnover[48]). Emerging evidence suggests associations between age acceleration and abundance of specific bacterial species,[49] while family history of cancer and presence of inflammatory bowel disease may also have an impact. These remain to be fully explored in relation to epigenetic aging and cancer risk.

Together, our findings demonstrate differential estimates of epigenetic aging by model, tissue type, and region of the colorectum and an impact of lifestyle. Our study underlines the need for careful consideration of model selection within study design and caution with data analyses and interpretation but demonstrates the potential for these models to provide insight into carcinogenic processes, where used appropriately.

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Author contributions

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Supplementary Material

Supplementary material is available at the *Journal of National Cancer Institute* online.

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Conflicts of interest

Adetunji Toriola (JNCI Associate Editor and co-author on this paper) and David Shibata (JNCI editorial board member and co-author on this paper) were not involved in the editorial review or decision to publish the manuscript. Cornelia Ulrich has cancer center director oversight over research funded by several pharmaceutical companies but has not received funding directly. The remaining authors declare no conflict of interest.

Data availability

All DNA methylation microarray data are available through Gene Expression Omnibus (accession codes: GSE101764; GSE131013; and GSE132804).

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