Shared genetic and epigenetic changes link aging and cancer

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Abstract

Aging is a universal biologic process that increases the risk of multiple diseases including cancer. Growing evidence shows that alterations in the genome and epigenome, driven by similar mechanisms, are found in both aged cells and cancer cells. In this review, we detail the genetic and epigenetic changes associated with normal aging and the mechanisms responsible for these changes. By highlighting genetic and epigenetic alterations in the context of tumorigenesis, cancer progression, and the aging tumor microenvironment, we examine the possible impacts of the normal aging process on malignant transformation. Finally, we examine the implications of age-related genetic and epigenetic alterations in both tumors and patients for the treatment of cancer.

Keywords

Aging; epigenetics; genetics; cancer; tumor microenvironment

Aging is a multifactorial, time-dependent biologic process that increases the susceptibility to multiple diseases including cancer [1]. Advancing age is one of the most studied risk factors for the development of most cancers, and it is projected that the total cancer incidence in the United States will increase by 30% from 2020 to 2040, at which point patients 65 years or older will account for ~69% of all new cancer diagnoses [2]. As

the global population ages, understanding the processes that drive normal aging and how they affect the development of cancer becomes critically important from both biologic and epidemiologic viewpoints. Aged cells and cancer cells seem fundamentally different since aged cells typically exhibit decreased rates of proliferation and energy production and cancer cells are typified by increased rates of proliferation and energy consumption. However, molecular changes found in aging cells are also drivers of tumorigenesis [3,4]. Importantly, many cancer-causing mutations can be found accumulating with age in normal cells and stem cells, and further, studies have shown that some of these are tissue specific [5,6]. A mathematical calculation of cancer risk based on lifetime accumulation of genetic damage in stem cells, in combination with extrinsic (smoking, UV damage) and intrinsic (heritable genetic changes) factors has been proposed [7]. Additionally, some genes expressed early in life may be beneficial, but later in life expression of those genes may have a detrimental effect, a phenomenon known as **antagonistic pleiotropy**, and it has been suggested that **senescence** is a prime example of antagonistic pleiotropy [8]. The switch from beneficial to tumor-promoting gene expression may depend on the **tumor microenvironment (TME)**. Understanding these age-related changes will provide opportunities to further elucidate the mechanisms through which cancers develop and highlight new therapeutic vulnerabilities for the treatment of cancer.

The aging microenvironment as a driver of genetic and epigenetic changes leading to tumorigenesis.

Having recently extensively reviewed age-related changes in the TME that drive tumor progression [9], in this review, we focus largely on the cancer cell-intrinsic genetic and epigenetic changes that are impacted by aging. However, it is critical to point out key microenvironmental factors that drive these changes (Figure 1). In the aging field, the oxidative stress theory of aging refers to the accumulation of damage in response to the accumulation of reactive oxygen and nitrogen species. These may be **endogenous**, derived from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, hydrogen peroxidase, and other oxidases, or **exogenous**, derived from tobacco, alcohol, and other pollutants [10]. Reactive oxygen and nitrogen species can interact with multiple types of molecules from lipids to carbohydrates and proteins, causing damage to these molecules that can result in cellular senescence. Further, reactive oxygen/nitrogen species can interact with and damage DNA, causing genomic instability, as well as transcriptional errors, and this is marked by the accumulation of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG), a product of DNA oxidation. Consequently, 8-oxo-dG has been shown to accumulate with age [11]. In order to counteract this damage, fibroblasts secrete molecules such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, but it has been shown that fibroblasts lose expression of these protective molecules over time leading to an accumulation of damage [12,13]. Additionally, the base-excision repair protein, APE-1/Ref1, which specifically deals with DNA damage wrought by oxidative stress is down regulated during aging. In addition to downregulation in fibroblasts, APE-1 in cancer cells can also be downregulated in response to age-related changes in secreted factors from fibroblasts. It has been shown that changes in Wnt signaling in cancer cells exposed to aged fibroblasts result in the loss of activity of

the microphthalmia transcription factor (MITF). MITF in turn does not transcribe *APE-1*, making the cancer cells susceptible to sustaining further damage [14].

Accumulation of DNA damage downstream of APE-1 can lead to cellular senescence, a cellular phenomenon that is linked to aging [15]. Senescence is a complex phenomenon, with important roles in tumor progression, and would require extensive review in and of itself [16], however, we summarize very briefly its importance here. Senescence is often initiated through a p16 or p53 mediated response to DNA damage, and results in cells going into a growth arrest. During aging, this DNA damage response can be induced by the dysfunction of telomeres, and the accumulation of chromatin marks, both of which are thought to maintain senescence through p53 [16,17]. Senescence is also accompanied by the secretion of various factors, such as IL-6, IL-10, interferons, chemokines, wnt ligands, and other cytokines, collectively known as the Senescence-Associated Secretory Phenotype (SASP) [18]. The SASP has been shown to both suppress tumor growth by inducing a paracrine senescence in tumor cells through an **inflammosome**-mediated mechanism, but also can paradoxically induce tumor progression [18]. The contributions of the immune microenvironment to senescence are summarized in Box 1. This is due to the fact that not only can senescence of tumor cells make them more resistant to therapy, since many therapies are targeted against rapidly proliferating cells, but also because signaling pathways induced by the SASP can be oncogenic [19].

It is also important to note that malignant cells can themselves impact normal cells, further propagating a genomically unstable microenvironment. For example, stromal cells near a tumor exist in an environment that may promote the accumulation of DNA alterations. Using single-cell multiomics sequencing, it was discovered that somatic copy number alterations (SCNAs) are present in fibroblasts, endothelial, and immune cells in both the TME and normal tissues of individuals with cancer [20]. Interestingly, the proportion of fibroblasts with SCNAs in close proximity to malignant cells was significantly higher than those residing in adjacent normal tissues. This suggested that cells in the TME may be genetically altered through a mechanism depending on proximity to cancer cells. Fibroblasts with SCNAs may interact with tumor cells to promote cancer progression, and this is an active area of ongoing research. Therefore, the effect on the microenvironment on genetic instability in cancer cells is a complex one - direct damage to initiated tumor cells due to oxidative stress and environmental factors, or through inflammatory pathways such as the SASP from neighboring cells. In turn, the malignant tumor cells can feed back to create further damage to normal cells in their immediate microenvironment. These combined changes, external and internal to the initiated cancer cell, drive increased genomic instability, ultimately leading to tumor progression.

Genomic instability and the accumulation of genetic alterations in aging cells

Normal human cells constantly replicate their genome and divide, increasing the risk of acquiring genetic alterations and DNA damage. DNA damage comes in many forms and includes single-stranded breaks (SSBs), double-stranded breaks (DSBs), alteration of

nucleotides, and the formation of bulky DNA adducts [21]. Most of these alterations will not lead to deleterious effects on cellular function and the majority of these alterations will be repaired by the endogenous DNA repair mechanisms found in normal cells. However, some changes to the genome can have far-reaching implications for cells during aging and in multiple diseases including cancer. For instance, unrepaired DNA alterations can impact the function of tumor-suppressor and oncogenes that in turn increase the risk of malignant transformation [22]. Analyses of the genomes from healthy cells have shown that the accumulation of somatic mutations in normal cells is directly correlated with the risk of developing cancer [23]. Many of the same mechanisms known to cause genomic instability in cancer cells have been implicated in the normal aging process [3], highlighting how changes during aging may enhance the risk of developing cancer with advancing age.

Changes in DNA repair pathways during aging

Unrepaired DNA alterations accumulate in an age-dependent fashion, suggesting that DNA damage response and repair pathways become less efficient with age [24]. Extensive evidence exists for age-related changes in multiple DNA damage response pathways. Declines in the activity, fidelity, or expression levels of components of the mismatch repair [25], base excision repair [26], nucleotide excision repair [27], non-homologous end joining (NHEJ) [28], and homologous recombination (HR) [29] repair pathways during aging have been reported in both human and animal models.

In the case of base excision repair, decreased efficiency with age has been linked with a reduction in Sirtuin 6 (SIRT6) levels. SIRT6 is a member of the **sirtuin** family of nicotinamide adenine dinucleotide^{+}-dependent enzymes that regulate chromatin signaling, metabolism, and aging (Figure 2) [30]. In a study of base excision repair in foreskin fibroblast from donors ranging in age from 20 to 64 years old [31], there was a significant decrease in base excision repair efficiency and a concomitant decrease in SIRT6 expression with age in a PARP1-dependent fashion.

Two major pathways, NHEJ and HR, serve to repair DSBs in human cells, and aged cells have lower DSB repair efficiency [32]. A study examining 50 eyelid fibroblast cell lines isolated from healthy donors ranging in age from 16-75 years old demonstrated an age-related decline in HR efficiency as well as NHEJ efficiency and fidelity in these cells [28]. In the case of NHEJ, mechanistic studies showed that decreased expression of multiple proteins that comprise both the classical and alternative NHEJ pathways (Figure 3) drove this phenotype. Decreased NHEJ and HR efficiency sensitized normal cells from older donors to ionizing radiation, a finding that has implications for older individuals exposed to radiation such as patients receiving cancer radiation therapy. Additionally, the NR4A family of nuclear receptors, which also participate in DNA double stranded break repair, are expressed at lower levels in a number of age-related disease including atherosclerosis and multiple cancers [33].

Aging, the accumulation of genetic alterations, and cancer risk

Somatic genetic alterations that are known drivers of tumorigenesis when found in cancers have been identified in the cells of people without cancer. Some of these alterations have been identified early in life. For instance, the leukemia-associated *AML1-ETO* gene fusion is present in cord blood in 1% of neonates, however, aging remains the strongest independent predictor for the presence of somatic mutations in normal tissues [34].

Population studies utilizing whole exome sequencing have identified recurrently mutated genes, including *TET2, DNMT3A,* and other genes known to be involved in the development of hematologic malignancies, in the peripheral blood of healthy, older individuals [35]. Clonal hematopoiesis, where a substantial proportion of mature blood cells arise from a single hematopoietic stem cell, was once thought to be rare, but has now been shown to be ubiquitous. Up to 95% of individuals aged 50-60 years old harbor leukemia-associated mutations found in cells from peripheral blood samples [36]. Not all mutations found in cases of clonal hematopoiesis affect genes known to increase cancer risk. Nonetheless, patients with clonal hematopoiesis have a tenfold increased risk of developing a hematologic malignancy, and even suffer adverse clinical outcomes including increased mortality even in the absence of carrying identifiable leukemia-associated mutations [37].

Somatic DNA alterations in normal tissues from solid organs have been well-described in the context of preneoplastic diseases and tumor-adjacent normal tissues [38]. Normal skin and skin cancers including melanoma are particularly illustrative of the link between DNA damage from environmental exposures and an age-related accumulation genetic alterations as drivers of tumorigenesis. Many mutations that act as drivers in melanomas are linked predominantly to ultraviolet radiation damage, and over time these errors accumulate in DNA after each cell division [39]. Ultradeep sequencing of 74 cancer-associated genes in biopsies of normal, sun-exposed skin showed the presence of somatic mutations in clonal patches [40] identified that 18-32% of normal skin cells harbored positively selected mutations in known melanoma-associated genes including *NOTCH1, NOTCH2, FAT1*, and *TP53*. These cells still maintained the functions required in the normal epidermis, showing that aged sun-exposed skin is a patchwork of evolving clones that may one day give rise to cancer.

More recent evidence has linked age as a key determinant of the mutational landscape in melanoma. Melanoma is a disease with many clinical subtypes that are often associated with specific oncogenic mutations. Mutations in **BRAF** and **NRAS** are found at high frequencies in cutaneous melanomas, and their presence is typically, though not always, mutually exclusive [41]. *BRAF* mutations affecting the V600E residue are found more frequently in younger patients [42]. Through the use of mathematical models to analyze genomic data from melanomas from patients across a wide age spectrum, it was shown that *NRAS* and *NF1*-driven melanomas exhibit a correlation between increase in UV damage and cell division mutations with age, but *BRAF*-mutated melanomas were associated only with UV damage [43]. It is conceivable that genetic changes incurred throughout the aging process may dictate the overall mutational landscape of solid tumors and the tumor microenvironment. Another study in head and neck cancers supported this hypothesis by

revealing distinct gene networks closely associated with migration and pro-growth signaling are recurrently altered in older patients [44]. Further study in this area is required to determine the generalizability of these findings to other cancer types.

Epigenetic alterations during aging

In addition to DNA alterations, epigenetic changes that do not alter an organism's DNA sequence are important contributors to the normal aging process [45]. As organisms age, there is a general loss of histone proteins, widespread chromatin remodeling, changes in histone modifications, and a change in the DNA methylation pattern throughout the genome [46]. These epigenetic alterations lead to distinct transcriptional changes associated with an aged phenotype. Here we will highlight a subset of the known age-related epigenetic changes.

Histone and heterochromatin loss during aging

DNA in human cells is wrapped around histone proteins to form nucleosomes. Each nucleosome is comprised of DNA wound around eight core histone proteins (H2A, H2B, H3, and H4, and nucleosomes) can then be organized into more complex three-dimensional structures known as chromatin [47]. The number of histones as well as changes that occur in chromatin structure affect the accessibility of DNA and are key elements in the regulation of gene expression and genome stability during DNA replication [48] (Box 2). Heterochromatin, a tightly packed form of DNA and histone protein complexes, is a key structural feature of eukaryotic chromosomes important for regulation of DNA repair, chromosome segregation during cellular division, and the restriction of lineage-specific gene expression [49].

One proposed model to explain the link between epigenetics and aging is the heterochromatin loss model. Multiple studies have supported the hypothesis that changes in histone expression and occupancy alter the structure of chromatin away from the tightly packed heterochromatin form in aged cells [50]. A study of replicative aging employing the use of micrococcal nuclease-DNA sequencing (MNase-seq) in budding yeast cells showed that nucleosome occupancy decreased by approximately 50% across the entire genome, leading to a rearrangement of nucleosome binding, widespread transcriptional activation, and genomic instability [51]. In fact, nucleosome occupancy is altered in aged mouse cells even in the absence of appreciable changes in canonical histone expression levels. Through the use of chromatin immunoprecipitation sequencing (CHIP-seq), it was demonstrated that aged mouse tissues from 29-month-old mice show a change in the H3 occupancy despite having similar overall H3 expression levels as tissue from 3-month-old mice. The changes in histone occupancy were associated with an increase in chromatin accessibility at pro-inflammatory genes [52], highlighting that age-related changes in chromatin organization lead to changes in cellular functioning that recapitulate aged phenotypes.

Changes in histone modification with aging

Post-translational modification (methylation, phosphorylation, ubiquitination, and acetylation, among others) of the N-terminal tails of core histones are important activators and repressors of transcription [53]. The balance of either activating or repressive modifications of histones has been shown to vary with age and is linked with lifespan [45]. The link between changes in global histone acetylation and aging is among the best understood. The addition of acetyl groups to lysine residues of histones by histone acetyltransferases (HATs) decreases the interaction between DNA and histones and activates transcription. Conversely, histone deacetylases (HDACs) act to remove acetyl groups from histones and repress transcription [54].

Histone H3 can be acetylated at lysine 9 (H3K9ac) and is then associated with active chromatin and aged cells [55]. In addition to its role in regulating base excision repair during aging, SIRT6 is a H3K9 deacetylase that has been shown to impact cellular longevity and survival through epigenetic mechanisms including a decrease in H3K9ac [56]. SIRT6 deficiency decreases lifespan [57], and SIRT6 overexpression leads to increased lifespan in mice [58]. SIRT6 also has deacetylase-independent functions that affect the epigenome. Depletion of SIRT6 results in altered telomere structure similar to those found in cells from patients with Werner syndrome, a premature aging syndrome [59]. SIRT6 can also promote heterochromatin silencing at retrotransposable elements. Specifically, SIRT6 can bind and post-translationally modify regulatory elements for Long Interspersed Element-1 (LINE-1) leading to suppressed transposition [60]. However, in both replicatively senescent human dermal fibroblasts and in 24-month-old mice, SIRT6 is depleted from LINE-1 regulatory sequences resulting in heterochromatin activation and transposition compared to young controls, events that increase the risk of genomic instability.

Chromatin remodeling

Chromatin undergoes extensive remodeling to support critical cellular processes including DNA replication, DNA damage repair, and transcription. These changes in chromatin structure are regulated by ATP-dependent chromatin remodeling complexes [61]. Despite the fact that aging and premature aging syndromes are characterized by changes in chromatin structure, the role chromatin remodeling complexes play in aging remains incompletely understood. Chromatin remodeling complexes implicated in the aging process include Mi2 (CHD-3/CHD-4). Depletion of the *C. elegans* Mi2 homolog, LET-418/Mi2, results in enhanced lifespan and stress resistance that was at least partially dependent on the longevity-associated DAF-16/FOXO transcription factor [62]. DAF-16/FOXO is also dependent on co-localization with chromatin remodeling SWI/SNF subfamily members at DAF-16/FOXO target promoters to activate genes associated with stress resistance and longevity [63].

Another developing field of aging research concerns changes in 3D chromatin structure and how it changes during aging. Using **Hi-C**, it has been shown that chromatin in human cells is spatially segregated into two compartments and further partitioned into topologically associated domains [64]. One of the first examinations of this structure in the context of aging came from a study of late passage cells from patients with the premature aging disease

Hutching-Gilford progeria syndrome. These cells exhibited a global loss of chromosome compartmentalization and disassociation of heterochromatin from the nuclear lamina [65].

Changes in DNA methylation during aging

Methylation of DNA is a widely studied epigenetic modification that has been well-characterized in the context of aging. DNA is methylated through the covalent linkage of a methyl group to the fifth position of the cytosine resulting in the generation of 5-methylcytosine (5mC) [66]. The presence of 5mC can interfere with site-specific binding of transcription factors which in certain contexts can lead to a repressive, constitutive heterochromatin state. Additional effects of DNA methylation on genome function, including at non-promoter/enhancer regions, are not necessarily associated with transcriptional repression [67], and their link to cancer and aging is a topic of continued research.

DNA methylation plays a key role in development where it can silence expression of unneeded genes in a tissue-specific manner [68]. During aging in mammals, there is a general decrease in DNA methylation across the genome, leading to activation of normally silenced genes and other DNA sequences including transposable elements [69]. In studies of identical twins, the patterns of DNA methylation loss diverge between genetically identical individuals over time, likely due to environmental factors or random errors in the transmission of DNA methylation during genome replication [70]. However, there are also specific regions of the genome that undergo changes in methylation patterns during aging that are reproducible across individuals. This loss of CpG methylation at transposable elements increases the risk of retrotransposition events, providing a link between age-related changes in the epigenome and alteration of the genome itself [69,71].

A decrease in the expression levels of the DNA methyltransferase DNMT1, which helps maintain genomic methylation stability, during aging may in part explain the progressive decrease in global DNA methylation with age [72]. *Dnmt1* haploinsufficient mice exhibit age-dependent decreases in bone mineral density and impaired learning and memory function without suffering a significant increase in mortality, suggesting that DNMT1 activity is involved in maintaining age-related physiologic health [72]. In addition to hypomethylation, aging has also been associated with site-specific hypermethylation at genes associated with development of age-related diseases [73,74].

These and numerous other studies have rapidly advanced our knowledge of the mechanisms that are responsible for the multitude of changes in the epigenome that occur with aging. However, a major outstanding question in the field is whether or not these changes directly impact longevity-associated genes and cause aging, or if the epigenetic changes are a consequence of genetic alterations of those same longevity-associated genes.

Epigenetic alterations in aging and their impact on tumorigenesis

The link between cancer and epigenetic dysregulation has been thoroughly explored and remains an important topic of research. Changes in epigenetic regulation of genes has been shown to increase expression of oncogenes, silence tumor suppressors, and drive

tumorigenesis [75]. Many of the same epigenetic alterations observed in studies of aging organisms have been implicated in the development and progression of cancer [76,77], helping illustrate a possible connection between age-dependent epigenetic changes and increased cancer risk in older individuals.

The comparison of changes in DNA methylation patterns during aging with those found in cancer cells further supports the interconnectivity between an aging epigenome and cancer (Figure 4). Just as hypomethylation is a major feature of aging cells, hypomethylation was one of the earliest described epigenetic changes in human cancers that has been reported across multiple tumor types [78]. In addition, site-specific hypermethylation in cancer is also implicated in tumorigenesis through the silencing of tumor suppressor genes. DNA methylation patterns in non-neoplastic gastric epithelia have an age-dependent increase in methylation of the promoters in multiple tumor suppressor genes including *LOX*, *p16/ CDKN2A*, *RUNX3*, and *TIG1*, suggesting that a subpopulation of normal cells that have already undergone changes in methylation may be more likely to undergo oncogenic transformation [79].

Perhaps the most-well known epigenetic link between aging and cancer is site-specific hypermethylation of developmental genes. This hypermethylation is characteristic of aging tissues and occurs predominantly at Polycomb-target sites that are associated with so-called "bivalent" chromatin – histones that are dually marked with both active and inactive modifications [80]. This same pattern of hypermethylation at bivalent chromatin domain promoters is also a key feature of multiple cancers, with a proposed model that aberrant gene silencing events that disrupt normal cellular homeostasis are stabilized as heritable gene silencing events in evolving abnormal clones that promote tumorigenesis [81].

A recent study, however, has shown that DNA methylation changes observed in aged cells and cancer cells may differ more than previously thought. This study found that aged cells showed a propensity toward global DNA hypermethylation and cancer cells showed strong bidirectional methylation changes compared to normal tissues, though a limitation of the study was a lack of ability to assay methylation at repetitive DNA regions [82]. Even when considering only differentially hypomethylated DNA sequences, there were surprising differences between aged and cancer cells when it came to the chromatin signature at areas of hypomethylation; genomic regions marked with the activating posttranslational histone modification H3K4me1 in aged cells, while in cancer, loss of DNA methylation was primarily associated with the repressive H3K9me3 modification. Furthermore, DNA hypomethylation in cancer was associated with heterochromatin regions while chromatin marks of DNA hypomethylation in aging were associated with enhancer regions, further complicating the link between DNA hypomethylation and tumorigenesis.

Impact of age-related epigenetic alterations for the treatment of older patients with cancer

While the majority of cancer diagnoses are made in patients over 65 years old, older patients are vastly underrepresented in clinical trials [83], there is a lack of robust clinical knowledge regarding toxicity risks in the average older patient with multiple co-morbidities

[84], and evidence suggests older patients have inferior disease-specific outcomes even when controlling for variables such as stage and patient performance status [85]. Finding important intersections between normal aging and the genetic and epigenetic alterations found in cancers may provide avenues for improvement in the care of older patients with cancer.

Aging, DNA methylation changes, and cancer risk have also been examined through DNA methylation clocks. These are mathematical models derived from epigenetic DNA methylation markers that correlate strongly with chronologic age. For example, individuals with "age acceleration," meaning their DNA methylation clock profile was similar to that of someone older by chronologic age, had an increased risk of developing pancreatic cancer [86]. Clocklike signatures in cancer may shape the overall mutational landscape of a tumor and thus affect a patient's ability to be treated with certain targeted therapies such as tyrosine kinase inhibitors. A recent analysis of the impact of clock-like mutational signatures in both melanoma and non-small cell lung cancer has highlighted another therapeutic implication of aging-related mutational signatures: response to immune checkpoint inhibitors (ICI). ICI, a class of immunotherapy that utilizes monoclonal antibodies that block immune checkpoints on CD8^{+} cytotoxic T-cells, has rapidly been adopted in standard of care treatment regimens for multiple cancers [87]. Analysis of whole-exome sequencing data from melanoma and non-small cell lung cancer tumors obtained from patients undergoing treatment with ICI identified the presence of an age-related clock-like mutational signature, characterized by enrichment of C→T mutations at NpCpG trinucleotides [88]. This was associated with lower tumor mutational burden, reduction in cytotoxic T cell tumor infiltration, increased regulatory T cells, and worse prognosis after treatment with ICI. While this data may help subtyping patients based on genomic data and guide therapy choices, further work is required to determine if an age-related mutational signature is the cause these unfavorable biomarkers and clinical outcomes after ICI treatment.

Given the importance of epigenetic alterations in cancer, drugs targeting epigenetic modifying pathways and proteins have been developed and successfully used in the clinic [89]. Notable examples include the DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi). A more recently appreciated intersection between the epigenetics of aging and cancer therapy is the effect of epigenetic alterations in the cells of the immune system and response to immunotherapy (Box 1).

Concluding remarks

Aging is an extremely complex biologic process. Here, we have focused on a subset of these changes with a focus on genetic and epigenetic alterations associated with age. Research over the past decades has shown that there is an age-dependent accumulation of genetic and epigenetic alterations in the genome. It has become increasingly apparent that the control of multiple longevity pathways and determinants of cellular function are regulated by these age-related genetic and epigenetic changes. In addition, a better understanding of these age-related changes has further strengthened the link between aging and cancer risk as many of the same alterations found in normal cells during aging are thought to be drivers of tumorigenesis in cancer cells.

Recent work has given us unique insights into the implications of age-related genomic and epigenomic alterations found in aged cells for the use of common cancer treatments including immunotherapies. Despite detailed understanding of many of the specific mechanisms and that cause these alterations, there is still a wide range of phenotypic variability among the older population. Continued genetic, epigenetic, and functional studies in both normal and cancer cells performed in different age groups are needed to not only add to our understanding of normal aging, but help refine and improve cancer treatment strategies for older patients (Outstanding Questions Box). Overall, age cannot be ignored as an important variable when designing pre-clinical experiments, or clinical trials.

Acknowledgements:

Glossary:

Antagonistic Pleiotropy
Refers to genes that confer fitness early in life, only to reduce fitness later in life, causing an aging phenotype

Tumor microenvironment
The collection of non-malignant cells (fibroblasts, immune cells, etc.), molecules, and blood vessels that comprise a tumor in addition to cancer cells

Endogenous sources of DNA damage
DNA damaging agents present inside of cells including reactive oxygen species and free radicals

Exogenous sources of DNA damage
Factors such as exposure to ionizing radiation, ultraviolet radiation, and chemicals including cigarette smoke that have potential to damage DNA

Senescence
A stable cell cycle arrest that can be triggered by various stimuli as well as developmental signals. It is a dynamic process in which cells remain viable, but have alterations in metabolic activity and gene expression [90].

Inflammasome
Multiprotein complex involving innate immune system receptors and sensors that regulate activity of inflammatory cysteine-dependent aspartate-directed proteases (caspases) after detection of pathogenic microorganisms or other danger signals in cells [91].

BRAF
A proto-oncogene mutated at high frequency in multiple cancers such including melanoma. The gene encodes the serine/threonine-protein kinase B-Raf which is involved in directing cell growth [92].

NRAS

A proto-oncogene frequently mutated in melanoma and other cancers that encodes the NRAS enzyme which is part of a family of G-like regulatory proteins involved in the normal control of cell growth through its GTPase activity [93].

Sirtuins

a family of protein deacetylases and ADP-ribosyltransferases involved in multiple cellular processes including metabolic and epigenetic regulation. Sirtuin family members have been implicated in regulating the mammalian lifespan, however the role of sirtuin family members in longevity is still disputed [94].

HI-C

A method to comprehensively detect chromatin interactions in the mammalian nucleus in which chromatin is crosslinked, digested, and re-ligated so that only DNA fragments that are covalently linked together form ligation products. This technique provides information on where in the genomic sequence chromatin interactions occur, but also where in the 3D organization of the genome they reside [95].

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BOX 1:

Age-related epigenetic and genetic changes in the tumor immune microenvironment

During aging there is a decline in immunity and the development of chronic low-grade inflammation (inflammaging) [96], resulting from the accumulation of both genetic and epigenetic alterations throughout life. The frequency and diversity of both peripheral circulating and tumor-infiltrating CD8+ cytotoxic T cells declines with age [97]. A recent study identified a clonal population of CD8+ T cells associated with inflammaging that have an exhausted-like phenotype, accumulated in tissues, and promote neighboring cells to adopt a senescence associated secretory phenotype through secretion of granzyme K [98], termed immunosenescence. These cells had a specific epigenetic signature that developed in response to an aged host microenvironment in *in vivo* studies. Multiple studies have highlighted that tumor DNA hypomethylation is associated with increased expression of immune checkpoint molecules or their immune-evasion promoting ligands in malignant cells [99]. Additionally, epigenetic changes in T cell populations may also have impacts on immunotherapy response. For instance, CD8+ memory T-cells, a subset that is exceptionally important for mediating successful tumor clearance, undergo changes in chromatin structure during aging that lead to less accessible DNA in regions known to be critical for response to NF-κB and STAT factors and may result in diminished immune responses [100].

Regulatory T-cells (Tregs) are also affected by aging-related epigenetic changes. Treg dysfunction can contribute to immunosenescence through enhanced suppression of effector T-cell response. Hypomethylation of an enhancer upstream of *FoxP3*, a major regulator of Treg function, is associated with aging in mice, and aging is associated with increased Treg numbers [101]. It is tempting to assume that with this increase in Treg number, older patients with cancer treated with immunotherapy would have a higher proportion of tumor-infiltrating Tregs in a melanoma model has demonstrated the opposite [102], suggesting that other changes in the tumor microenvironment in older patients may impact Treg trafficking and function. This is further supported by data from a study of patients with head and neck squamous cell carcinoma showing older patients had increased Tregs in the peripheral blood but decreased tumor-infiltrating Tregs [97]. Clinical experience with the use of ICI in older patients has shown ICI to be highly effective in this population [9]. Finally, myeloid-derived suppressor cells (MDSCs), implicated in tumorigenesis, metastasis, and alteration of tumor immune surveillance, increase in frequency with age, possibly due to alterations in epigenetic regulation that favor myeloid differentiation of hematopoietic precursors [103].

BOX 2:

Histone variants

In addition to the loss or post-translational modification of core histones with aging, core histones are often replaced with variant histones in aging organisms [71]. Histone variants differ in primary sequence compared to their canonical counterparts and can have unique effects on the regulation of gene expression. For instance, the H3 histone family has seven variants in humans [104]. In contrast to the canonical H3.1 and H3.2, the H3.3 variant is incorporated in the genome in a replication-independent manner and is the major form of H3 found in the chromatin of senescent human cells [105]. H3.3 has been shown to accumulate during aging in mouse somatic tissues to the point of nearly completely replacing the core H3 histone by the time the mice were 18 months old [106]. Furthermore, H3.3 levels increase in human brain tissue over the first decade of life, though levels remained stable across individuals who were 14-72 years old [107]. Functional effects of H3.3 accumulation during aging was demonstrated in *C. elegans* where H3.3 positively regulated the lifespan of long-lived through activation of diverse lifespanextending signaling pathways, and lack of H3.3 results in a much shorter lifespan [108], suggesting that its accumulation may be a key feature of successful normal aging.

Outstanding Questions

1. Despite the increased understanding of the shared hallmarks between aging and cancer, direct links that will ultimately affect how cancer is treated remain active areas of investigation.

2. Do epigenetic in longevity-associated changes in aged cells directly cause aged phenotypes, or are these epigenetic changes driven by the accumulation of genetic alterations?

3. How do age-related changes in non-malignant cells in the tumor microenvironment, such as fibroblasts, alter the behavior of cancer cells and other components of the TME?

4. Can aging-related genetic and epigenetic changes be targeted or reversed to decrease the incidence of cancer in humans?

5. How can clinical trial participation by older patients with cancer be increased to enable better study of the link between aging, cancer, and cancer therapy?

Highlights

Multiple changes in genome stability and epigenetic regulation are shared between both biologic aging and cancer development, providing further evidence behind the mechanisms of the age-related increase in cancer incidence.

Age-related changes in the tumor microenvironment have been identified as key contributors to cancer progression and response to targeted and immunotherapies. Older patients are underrepresented in clinical trials, possibly limiting generalizability when considering the demographics of study populations.

Further study of aging and cancer will improve highlight new targets for cancer therapy and improve patient outcomes.
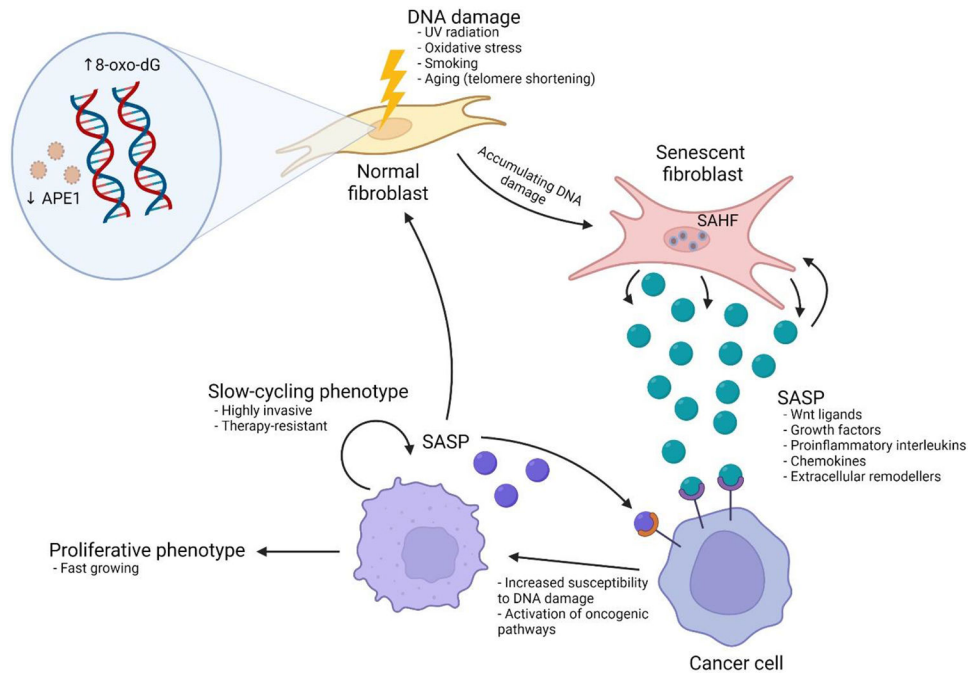
Figure 1. Age-related changes in non-malignant cells in the tumor microenvironment alter cancer cell behavior.
Fibroblasts are a principal non-malignant component of the tumor microenvironment. As fibroblasts are exposed to sources of DNA damage including UV radiation, oxidative stress, and the aging process, and this accumulating damage is marked by the presence of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG). In addition, the base-excision repair protein APE-1 is downregulated with age, further increasing the susceptibility to DNA damage. These fibroblasts can undergo cellular senescence. Senescent cells are characterized by the presence of senescence-associated heterochromatin foci (SAHF), specialized domains of heterochromatin that contribute to the silencing of proliferation-promoting genes and adopt a senescence-associated secretory phenotype (SASP) which has been shown to promote tumor growth by increasing cancer cell susceptibility to DNA damage and activation of oncogenic signaling. The end result can either be paracrine senescence of cancer cells, though the promotion of a slow-cycling cancer cell phenotype is associated with highly invasive, therapy-resistance cells, or enhancement of cancer cell proliferation.
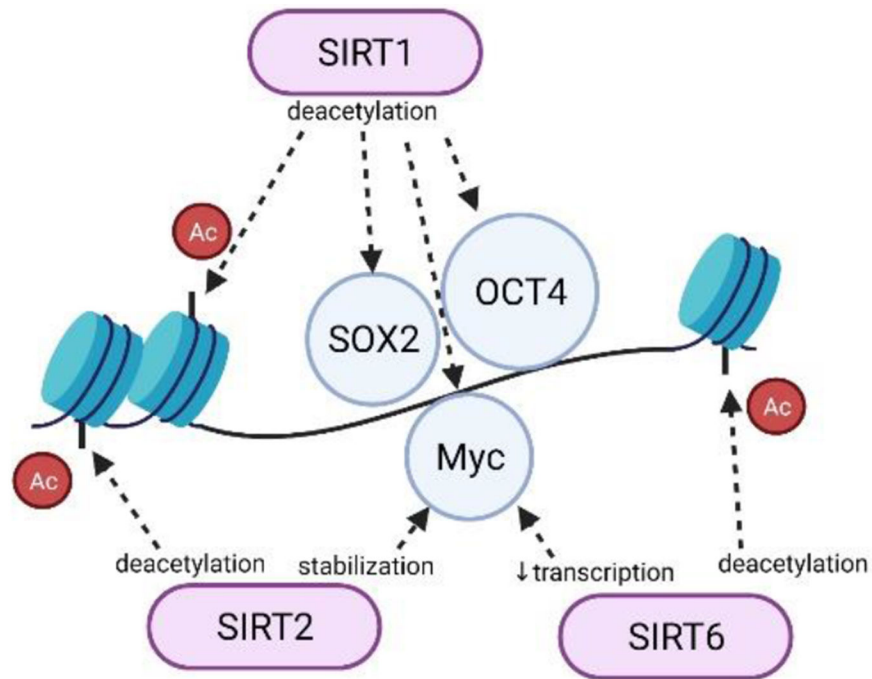
Figure 2. Sirtuins, histone modifications, and the regulation of chromatin and gene transcription.
SIRT1, SIRT2, and SIRT6 are involved in regulating chromatin remodeling. SIRT1 can deacetylate H3K9, H3K56, H1K26, and H4K16. It can also deacetylate the transcription factors SOX2, Myc, and Oct4. SIRT2 deacetylates H3K56, H4K16, and H3K18 and can stabilize Myc through alterations in Myc ubiquitination. SIRT6 deacetylates H3K9 and H3K56 and can repress the transcription of MYC.
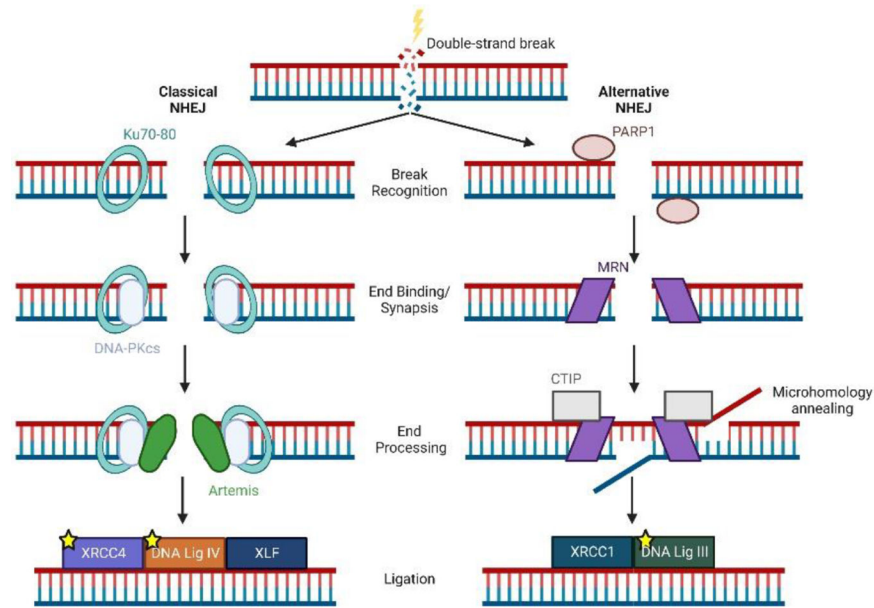
Figure 3. Classical and alternative nonhomologous end-joining (NHEJ) pathways.
After the formation of a double-strand break, the classical (left) or the alternative (right) nonhomologous end-joining pathways repair DNA damage through break recognition, end binding/synapsis, end processing, and final ligation of DNA. Proteins marked with a yellow star have been shown to have decreased expression during aging.
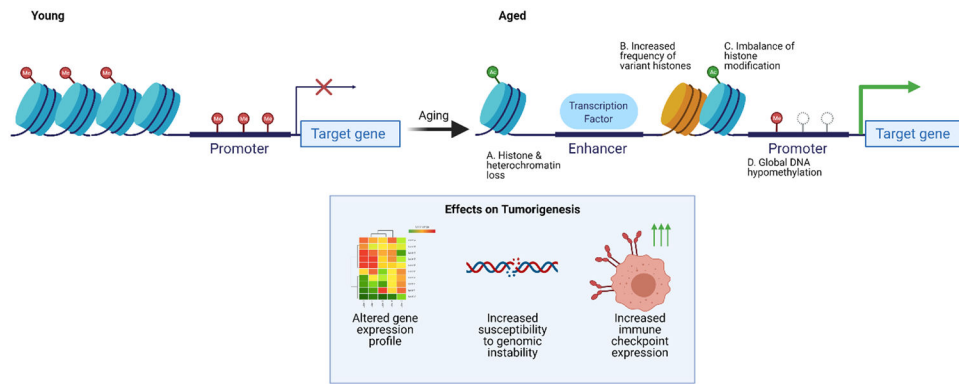
Figure 4. Age-related epigenetic changes and their effects on tumorigenesis.
(A-D) Aging is characterized by multiple alterations in the epigenome including (A) loss of histones and heterochromatin, (B) an increased frequency of non-canonical, variant histones, (C) a change in the balance of activating and repressing histone modifications, and (D) global DNA hypomethylation among others. These changes have been implicated in tumor growth and progression through the alteration of gene expression, genomic instability, and an increase in immune checkpoint molecule expression in cancer cells (inset box).