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Concise Review: The Epigenetic Contribution to Stem Cell Ageing: Can We Rejuvenate Our Older Cells?

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ABSTRACT

Although certainly one of the most recognizable characteristics of human biology, aging remains one of the least understood. This is largely attributable to the fact that aging is both gradual and inherently complex, with almost all aspects of physiology and phenotype undergoing steady modification with advancing age. The complexity of the aging process does not allow for a single all-encompassing definition, yet decades of study using diverse systems, methodologies, and model organisms have begun to build a consensus regarding the central physiological characteristics of aging. Indeed, such studies have shown that the process of aging is invariably accompanied by a diminished capacity to adequately maintain tissue homeostasis or to repair tissues after injury. When homeostatic control diminishes to the point at which tissue/organ integrity and function are no longer sufficiently maintained, physiologic decline ensues, and aging is manifested. Inadequate organ homeostasis indicates possible dysfunction of tissue-specific stem cells. Several mechanisms have been postulated to account for age-related cellular changes; however, increasing literature evidence suggests that age-related changes to the epigenome make a major contribution to the aged phenotype. In this review, we discuss the evidence for epigenetic contributions to tissue-specific stem cell ageing. STEM CELLS *2014;32:2291–2298*

AGEING AS A RESULT OF DECLINING STEM CELL FUNCTION

Many of the pathophysiological conditions afflicting the elderly, such as anemia, sarcopenia, and osteoporosis, suggest an imbalance between cell loss and renewal. The fact that homeostatic maintenance and regenerative potential of tissues wane with age has implicated stem cell decline as a central player in the aging process. However, the degree to which aging is attributable to stem cell dysfunction or instead reflects a more systemic degeneration of tissues and organs will likely differ substantially between different tissues and their resident stem cells. Nevertheless, mounting evidence points to stem cells as an important contributing factor to at least some of the pathophysiological attributes of aging in a number of different tissues. Adult stem cell populations maintain highly differentiated but short lived cells such as blood, intestinal epithelium cells, and sperm throughout life. Upon division of stem cells, daughter cells must either self-renew to preserve stem cell identity or commit to differentiation. The balance between stem cell self-renewal and differentiation is critical to tissue homeostasis, with disruption of this balance leading to tumorigenesis (caused in some cases by stem cell overproliferation) or tissue degeneration (caused by stem cell depletion). A decline in the function of adult stem cells and their supportive niches has been proposed to contribute to tissue ageing, although the underlying mechanisms remain enigmatic [1, 2]. Tissue ageing has been proposed to have arisen as a tumor suppressor mechanism [3–6], in which tumor suppressor activity reduces stem cell function in later stages of life, preventing tumorigenesis but reducing tissue regenerative capacity. However, the cellular and molecular basis of such phenomena are poorly understood.

THE CAUSES OF AGEING ARE MULTIFACTORIAL

Why should organ and tissue homeostasis decline with age? The causes of this problem are still unclear and are without doubt multifactorial in origin but current evidence supports the involvement of three major contributing factors:

Progressive Accumulation of Macromolecular Damage

Efficient cell function is dependent upon the structural integrity of its DNA and the protein products synthesized using the information it

encodes. The genome is a fragile and highly conserved structure that accumulates a wide range of damaging alterations with age, despite continuous surveillance and repair [7–9]. The accrual of genomic defects can affect cellular function on many levels. For example, mutations in coding regions of DNA can cause abnormal protein expression or function, and chromosomal translocations and rearrangements can result in apoptosis, tumor formation, or senescence [10]. In addition, the shortening of telomeres during mitosis (or as a result of oxidative damage to the telomeric DNA) has been highlighted as a significant contributor to the onset of senescent arrest of mitosis [11, 12]. Consistent with a role for DNA damage in ageing, a variety of specialized DNA repair pathways have been linked to the ageing process. These include the removal of damaged bases or base adducts and the repair of the resulting single-stranded (ss) DNA lesions as well as the repair of DNA double strand breaks (DSBs). An association between DSBs and ageing is seen in a variety of genetic mutants. A prominent example is a defect in the human WRN protein. WRN is a member of the RecQ family of DNA helicases and is involved in several aspects of DNA metabolism, including DNA replication, maintenance of telomeres, and DNA repair [13]. WRN mutations cause a dramatic increase in DSB-related genomic instability, resulting in Werner syndrome, a progeria with onset in middle age that mimics many aspects of normal ageing including atherosclerosis, diabetes, and dramatically aged skin by age 40 [14]. Other progeric conditions have been described such as Hutchinson-Gilford progeria which results from mutations in the *LAMIN A* gene leading to structural dysfunction of the nuclear membrane which in turn enhances genomic instability [15]. There are other factors that may contribute to age-related cellular dysfunction such as the accumulation of mis-folded proteins [16, 17], accumulation of lipid oxidation by-products [18, 19], and proteins subjected to damage by reactive oxygen species (ROS); however, these are more likely to impact the functions of postmitotic cells while those that retain the capacity to divide may be able to reduce the impact of these types of damage.

Dysregulation of Epigenetic Mechanisms of Genome Control

The gene expression profiles of certain types of somatic cell are known to change with chronological age of the organism from which they are obtained. Murine hematopoietic stem cells (HSCs) show consistent alteration in the expression level of a large number of genes (1,500 upregulated and 1,600 downregulated in older mice) many of which are central to genome stability and chromatin regulation. Genes involved in the latter are mostly downregulated [20]. This results in declining hematopoietic function despite increased overall numbers of phenotypic HSCs in older mice. The most obvious alteration in function is the increasing tendency for HSCs to differentiate down myeloid in preference to lymphoid lineages [21]. Age-related transcriptional changes have been identified in many other somatic cell types [22]. The driving force behind these epigenetic changes is not yet known but all eukaryotes experience changes in chromatin organization with age [23]. It is well documented that aging cells subtly change their patterns of DNA methylation since cells undergo global hypomethylation while selected genes become progressively hypermethylated and, potentially, permanently silenced [24, 25]. Many genes affected by these changes are oncogenes or tumor suppressors; hence, DNA methylation loss (or gain) has frequently been linked to the development of cancer; however, there is substantial evidence to suggest that DNA methylation of specific gene promoters may also contribute to age-related alteration of the transcriptome in specific cell types [26]. Post-translational modifications such as the acetylation or methylation of specific lysines and/or arginines in the N-termini of the histone proteins integral to the structure of the nucleosome are established genome wide in patterns specific to individual types of cells [27, 28]. These patterns are created during embryonic development and are a further mechanism by which only the genes required for the functional phenotype of a particular type of cell will be allowed to undergo transcription while other genes that are surplus to this need will be repressed via the formation of heterochromatin which cannot be easily accessed by the transcriptional machinery. Histone modifications such as methylation, acetylation, and ubiquitination are known to change with age [29–31] which may contribute to the associated transcriptomic dysregulation. Literature evidence suggests that trimethylation of histone H3 at lysine 27 (trimethyl H3K27) undergoes a genome wide age-related increase in adult stem cells [32]. This may result from global decreases in the expression of proteins needed for chromatin maintenance or it may be the cell's response to changes in DNA methylation since this is known to be linked to the types of post-translational modification present on specific N-terminal tail amino acids of histones near the 5-methylcytosine modified CpG islands [33–35]. It is also possible that the alterations of both DNA methylation and histone modification patterns are simply the cell's response to accumulated damage in its genome (DNA mutations, unrepaired strand breaks, and oxidative lesions). If this is true, the resulting epigenetic modification pattern should not be reversible since it would be generated solely by changes in the underlying DNA sequence.

Age-Related Decline of Mitochondrial Function

Mitochondria provide the cell with the larger part of its ATP generating capacity. Although different types of cells use the oxidative phosphorylation system to different extents**,** a wealth of experimental data suggest that mitochondrial dysfunction may be a significant contributor to the progression of ageing which led to the formulation of Harman's theory that ageing is caused by the accumulation of damage resulting from ROS [36, 37]. ROS are a by-product of oxidative phosphorylation which arise in the mitochondria and can damage their smaller and less well protected genomes. The majority of genes contributing to mitochondrial function are encoded by the nuclear genome but important components of the electron transport chain arise from the 16 kb circular mitochondrial genome which does not have DNA repair system equivalent to those operating on nuclear DNA [38]. It has been estimated that mitochondrial genomes accumulate mutations at least 10× faster than nuclear genomes typically as point mutations or large deletions [39] and although mitochondria typically have multiple genome copies, such mutations are thought to disrupt the oxidative phosphorylation capacity once a threshold level of mutated genomes is reached. The contribution of this to the general ageing phenomenon awaits confirmation but there are several tissues

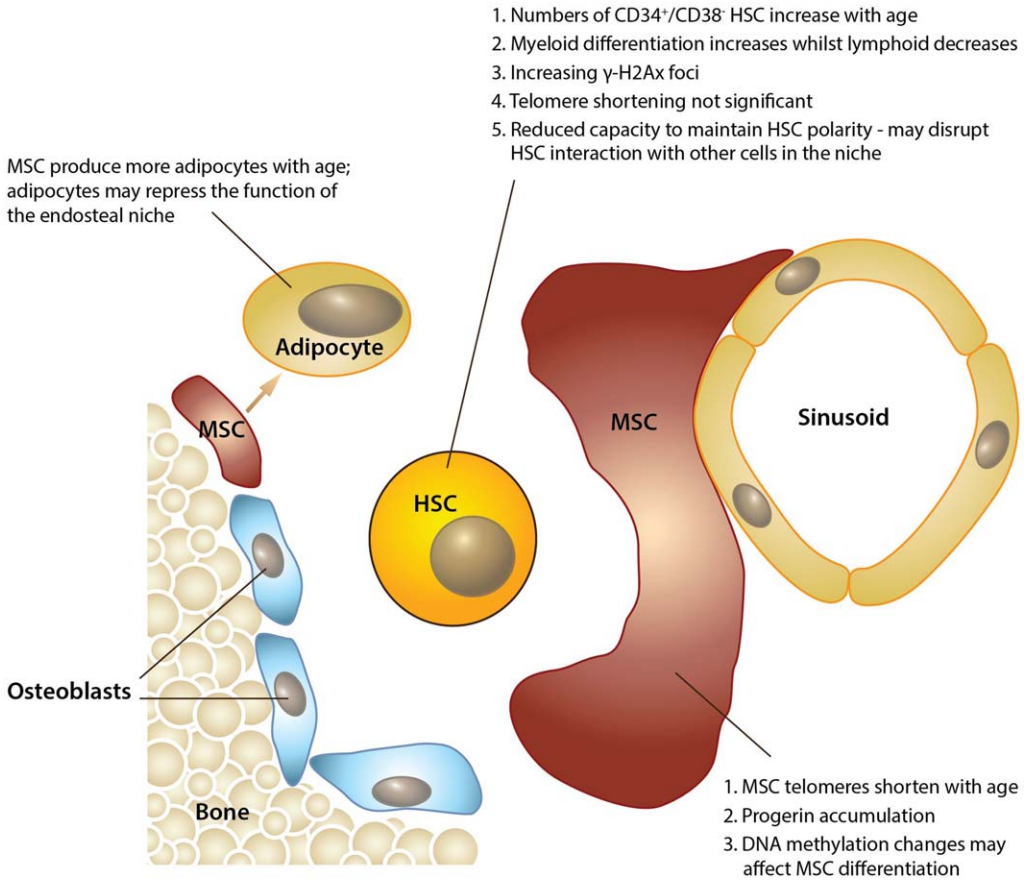

Figure 1. Summary of the age-related changes occurring in the HSC niche. HSC numbers do not decrease with age but these cells show a number of functional differences to their youthful counterparts; however, it is not yet clear if these differences are due to cell intrinsic changes or the influence of age-related change in the cells comprising the microenvironment. Abbreviations: HSC, hematopoietic stem cell; MSC, mesenchymal stem cells.

(brain, muscle, and colon) in which respiratory deficient cells harboring clonal expansions of mutant mtDNA may be abundant enough to impact the function of the whole organism [40, 41].

Do Tissue–Specific Stem Cells Accumulate Macromolecular or Epigenetic Changes with Age?

Hematopoietic Stem Cells

Most studies of the effects of ageing on adult stem cells focus upon the HSCs and the age-related changes they undergo are well documented. We have attempted to summarize the age-related changes affecting HSCs in Figure 1. HSC numbers do not decrease with age rather, the proportion of CD34+/CD38− or Lin−/CD34+/CD38−CD90+/CD45RA− increases in the elderly bone marrow of humans and mice, respectively [42, 43], but these cells show a number of functional differences to their youthful counterparts. The increasing preference for myeloid over lymphoid differentiation has already been noted [21] but why this should occur is not completely understood. One suggestion is that the relative percentage of myeloid committed progenitors increases with age at the expense of lymphoid committed cells although we do not know if such bias is due to an intrinsic change in the properties of HSC or if it simply arises because HSC that were committed to a myeloid fate earlier in the life of the individual become more numerous. The first possibility is the basis of a clonal alteration model [44] in which a homogenous population of HSCs gradually differentiates into cells that are more suited to generate myeloid cells. The alternative model implies a population shift within a heterogeneous HSC population to favor myelogenesis; in short distinct classes of HSC biased toward myeloid differentiation seem to proliferate better and accumulate in the bone marrow of older individuals. There is increasing evidence to support such a population shift [45, 46] but why the myeloid biased HSCs should be selectively expanded during ageing is not clear but both of these models could be influenced by the accumulation of macromolecular or DNA damage or alterations to the epigenetic control of gene expression with age.

There is only scant evidence for the accumulation of the products of macromolecular damage in HSC. Materials such as lipofuscin seem to accumulate preferentially in terminally differentiated, postmitotic cells and accordingly lipofuscin granules are observed in several human tissues particularly the myocardium, skeletal muscles, brain, liver, kidney, and gastrointestinal tract [17, 18]. The impact of such heterogeneous material is highlighted by the special case of the retina in which lipofuscin is associated with age-related macular degeneration [47], however while there is some evidence of lipofuscin accumulation in erythroleukemia cells treated with

adrenergic receptor agonists [48], there is little evidence suggesting that more primitive hematopoietic progenitors/stem cells are subject to such changes.

The evidence for accumulation of DNA damage in the nuclear genomes of HSCs is more convincing but even so, some conflicting data are reported. The repopulating capacity of human HSC deteriorates during long-term repopulation experiments in which lineage negative CD34+CD38− HSC were transplanted into immunodeficient mice. Serial transplantation of this population of cells was observed to upregulate the expression of cell cycle inhibitors $p16I^{NK4a}$, $p14^{ARF}$, and $p21^{CIP1}$ suggesting the accumulation of DNA damage. Accordingly, Lin−CD34+CD38− cells harvested from the serially transplanted mice exhibited multiple γH2A.X (marker of DSBs) foci and upregulation of markers indicating activation of the DNA damage response pathways such as expression of ATM, p53 binding protein, and checkpoint kinase 2. The hypothesis put forward to explain this was that cell proliferation accompanying hematopoietic repopulation may have generated ROS that lead to DNA damage. This was supported by the observation of a gradual but significant increase in ROS levels during serial transplantation [49]. Similar observations in Lin−CD34+CD38− cells from the bone marrow of elderly humans (72–84 years) showed greater incidence of γH2A.X foci than those from younger donors. As expected, HSCs from older individuals showed a reduced repopulating capacity compared to younger. Against a background of seemingly increasing DNA damage accumulation, one might expect to observe an age-related loss of telomeric DNA but the evidence for this during HSCs ageing is less convincing. The telomeres of Telomerase knockout mice shorten more rapidly than those of their wild-type counterparts; however, it is only the third generation of such animals that demonstrates critically short telomeres in their HSCs that have been linked to a premature hematopoietic ageing in this model system. It is not clear that HSC telomeres shorten sufficiently during a human lifespan to result in the accumulation of senescent HSC.

Accumulation of mitochondrial mutations in HSCs has also been observed by some workers [50] but it is not clear if this affects the functionality of HSC. Some reports suggest that mice carrying a proofreading defective mtDNA polymerase show some characteristics of premature HSC ageing but this only seems to restrict the differentiation ability of some types of progenitor cells that arise from HSCs [51]. These results show that intact mitochondrial function is required for appropriate multilineage stem cell differentiation, but argue against mitochondrial DNA mutations per se being a primary driver of somatic stem cell aging. Moreover, defining a clear relationship to donor age has been complicated by the fact that not all young donors have low levels of mitochondrial mutations suggesting that CD34+ cells accumulated mutations at all stages of growth.

Significant changes are seen in the polarity of HSC during ageing [52, 53]. Cells can be described as polarized when some or all their organelles are distributed in a nonsymmetrical manner. One of the best examples of this behavior is found in the actin filaments and microtubules that comprise the actin cytoskeleton. These structures are organized primarily by the centrosome (aka the microtubule organizing center) and since the microtubules are assembled outwards from the centrosome, polarity becomes fixed. Several other proteins are involved in establishing cell polarity, such as the partition protein PAR, Crumbs, and Scribble and these are often localized to their correct subcellular positions through the Golgi apparatus [54]. The precise impact of such polarity on HSCs function is still not completely clear but there is increasing evidence to suggest that HSCs polarity regulates the outcome of stem cell division to either symmetric or asymmetric divisions. Moreover, establishment of polarity seems to be important for correct integration of HSC into their niche. Aged HSCs show alterations in the distribution of the cell polarity makers tubulin and numb [55] implying a reduced capacity to maintain polarity. This correlates with the functional decline of HSC during ageing and may be linked to elevated activity of Cdc42 [56]. In addition, the findings that ageing in tissues other than the hematopoietic system seems to be associated with elevated Cdc42 expression and overexpression of Cdc42 seems to induced a type of premature ageing syndrome in mice support the possible involvement of this factor. Interestingly, pharmacological inhibition of Cdc42 activity appears to rejuvenate HSC functionality [57, 58] and restores epigenetic factors such as the spatial distribution of histone H4 lysine 16 acetylation to that found in HSCs from young donors [53] but the reasons behind the changes in polarity protein levels and Cdc42 are unknown.

Does Epigenetic Change Contribute to Stem Cell Ageing?

Epigenetic changes in HSCs as a function of age have been recorded by other workers. To identify transcriptional changes in aged HSCs that correlate with their known functional deficits, Chambers et al. examined the expression of 14,000 genes using microarray analysis of HSCs isolated as a c-kit-positive, lineage-negative, Sca1-positive side population and found that 1,600 genes that were upregulated at 21 months of age, and 1,500 that were downregulated [20]. Most of the genes that were consistently upregulated in aged HSCs belonged to categories previously linked to ageing in general such as nitric oxide-mediated signal transduction systems, protein folding, and inflammatory responses; however, genes undergoing substantial downregulation with age tended to be those involved in the preservation of genomic integrity such as chromatin remodeling and DNA repair. For example, the SWI/SNF-related chromatin remodeling genes Smarca4 and Smarc1b plus three histone deacetylase genes (*Hdac 1*, *5*, and *6*) and DNA methyltransferase (Dnmt3b) underwent significant downregulation with age. Furthermore, many of the genes were physically clustered together in specific genomic loci that changed coordinately with age suggesting either a loss of transcriptional silencing/activation at such loci or the preferential accumulation of DNA damage in these regions. While the occurrence of DNA damage such as point mutations and DSBs is probably random, it is not impossible that the effectiveness of DNA repair may be regulated by the chromatin environment of specific genomic loci (i.e., some regions may be repaired more efficiently than others), which may lead to some regions acquiring higher mutational loads than others. This could account for locus-specific age-related transcriptional differences; however, higher mutation rates are normally observed for intergenic noncoding DNA so the data generated by Chambers et al. are thought to support an epigenetic view of HSCs ageing. Analysis of a broad range of DNA methylation data from

healthy tissues, cancer tissues, and cancer cell lines has suggested that levels of methylated DNA present in a number of tissues may be a reasonable marker of the molecular age of the cells from which the tissue is built [26]. This study extends the observations of several investigators showing preferential age-related hypermethylation of CpG islands but it is interesting to note that embryonic stem cells and induced pluripotent stem cells do not seem to increase levels of DNA methylation at these same CpG islands regardless of the number of population doublings they undergo. Moreover, Horvath's data suggest that epigenetic reprogramming needed to generate induced pluripotent stem cells (iPSC) (reviewed in [59]) can reset the "epigenetic clock" back to zero. If changes to the epigenotype of a cell make a significant contribution to the development of its aged phenotype, then one might speculate that reversal, or otherwise alteration of such an aged epigenotype might reverse the age-related changes. Generation of chimeric mice by injection of iPSC into blastocyst stage embryos followed by implantation into pseudopregnant females can be used to produce "young" animals in which substantial contributions to several tissues (including the hematopoietic system) arise from the injected iPSC. A study by Wahlestedt et al. [60] described the generation of chimaeras from iPSC generated from Lin-c-Kit+CD45.1+ hematopoietic progenitor cells enriched from young and old C57BL/6 mice. The distribution of hematopoietic lineages derived from the injected iPSC was remarkably similar to those derived from the endogenous blastocyst regardless of whether the iPSC were derived from Lin-c-Kit+CD45.1+ cells of old or young mice. The inference from these data was that the myeloid skewing associated with HSCs ageing was reversed by reprogramming aged HSCs to iPSC. Similarly, the numerical increase in phenotypically defined HSC found in aged mice did not occur in the iPSC derived chimaeras and the ability of their bone marrow mononuclear cells to reconstitute lethally irradiated recipient was similar to that obtained from young HSC.

The Wahlestedt et al. study is interesting but raises several questions. The iPSC were derived from a heterogeneous population of hematopoietic progenitors not all of which will have accumulated identical levels of age-related changes (both in their nuclear and mitochondrial genomes), so it is conceivable that individual iPSC clones could have arisen from HSC that had escaped the accumulation of high levels of age-related change. HSC generated in chimaeras derived from such iPSC might be expected to show more "youthful" characteristics therefore one could not conclude that accumulation of damaged DNA or epigenetic dysregulation or change is a significant causative factor in the ageing process. This question may be resolved if iPSC can be generated from truly aged HSC enriched from the bone marrow of older donors. If mice produced from such iPSC by blastocyst injection show a typically young distribution of hematopoietic lineages, this will be a more robust demonstration of the reversal of age-related change using epigenetic reprogramming.

Other Types of Tissue–Specific Stem Cells

Mesenchymal Stem Cells

Mesenchymal stem cells (MSC) are adult tissue committed stem cells found in the bone marrow and other tissues that are capable of mobilizing, proliferating, and committing to terminally differentiated cell types such osteoblasts, adipocytes, chondrocytes, tenocytes, and myocytes [61, 62]. It has also been noted that MSC may be capable of differentiating into other lineages such as neurons, glial cells, and hepatocytes [63–65]. The ability to generate these two cells types coupled to their anatomical location in the bone marrow indicates that MSC may be a key component of the niche that supports and controls the activity of HSCs. MSC are also thought to differentiate into cells of cartilage and tendon but their most widespread contributions are thought to involve the production of interstitial stromal cells and fibroblasts. Published evidence, although sometimes contradictory, suggests that MSC functionality changes with donor age; primarily this involves alterations in their differentiation capacity namely reduced myogenic and osteogenic differentiation coupled to enhanced adipogenesis. These alterations in differentiation ability seem to depend on anatomical location; for example, the effectiveness of the hematopoietic niche may be diminished by reducing MSC differentiation to osteoblasts in favor of adipocytes. Abrogation of adipogenesis by inhibition of the peroxisome proliferator-activated receptor-γ enhances the engraftment ability of transplanted hematopoietic precursors [66] and supports the hypothesis that adipocytes exert a repressive influence on the hematopoietic niche. Conversely, MSC populations in the vasculature (also known as pericytes) show an increasing propensity to develop along osteogenic lineages which may contribute to ectopic calcification of the arteries and heart valves [67].

Why such changes occur is unclear. First, although MSC telomere lengths decrease with donor age, they are still partly maintained in adults suggesting that the telomerase enzyme complex is active in MSC even if only transiently [68–70]. Progeric conditions arising from *LAMIN A* (*LMNA*) mutations such as Hutchinson-Gilford progeria show extensive degeneration of the mesenchymal tissues [71, 72] suggesting that *LMNA* mutations have a significant impact on MSC function. Ectopic expression of *LMNA* and its alternative splice homolog Progerin induces MSC dysfunctions that are similar to those observed in MSC isolated from Hutchinson-Gilford progeria patients by activating downstream effectors of the Notch signaling pathway resulting in enhanced osteogenic differentiation and reduced adipogenesis [73]. Several lines of evidence have shown that Progerin accumulates with age due to alternative splicing; however, it is not clear that the very low expression of progerin in normally aged cells has any physiological relevance to the ageing process especially since progerin expression seems to be linked to progressive telomere damage and an inverse correlation between progerin expression and telomerase activity has been noted [74]. Activation of the cryptic splice site in the *LMNA* gene needed to generate progerin is thought to rely on changes in the control of alternative splicing processes; extensive changes in alternative splicing of many genes, including *LMNA*, occurs as telomeres shorten and cells approach senescence so it is possible that the low levels of progerin accumulating during normal ageing simply reflect the presence of small numbers of senescent cells normally found in the body. Why such changes in alternative splicing occur is unclear but if they are linked to telomere length and/or telomerase activity, then the apparent partial maintenance of telomere lengths in MSC might be expected to reduce the impact of progerin on MSC functional decline. An exciting possibility is that reprogramming

somatic cells back to a pluripotent state may "reset" progerin expression levels back to that associated with youthful tissues [75]. These data suggest that some characteristics of fibroblasts obtained from older donors (such as nuclear morphology abnormalities and decreasing levels of heterochromatin markers) are reset to youthful levels by generation of iPSC, however given that such iPSC were derived from heterogeneous populations of fibroblasts from such donors, there is still the possibility that individual iPSC clones could have arisen from a fibroblast that had escaped the accumulation of age-related changes.

There are also data showing that MSC can accumulate DNA damage during extended in vitro culture which may explain the changes in differentiation capacity observed in this system (e.g., increased adipogenesis) [76–78], there is little evidence to suggest that DNA damage accumulation either occurs or at least has a significant functional effect in vivo. Nevertheless, MSC from older donors are different to those from young. Long-term in vitro culture of bone marrow MSC produces consistent gene expression changes that correlate with significant alterations in DNA methylation present in the CpG islands of the promoters of the affected genes [79] but moreover, these epigenetic changes are remarkably similar to those observed in MSC derived from the bone marrow of older individuals. Quite why this epigenetic change should occur is not clear at present. Some workers have speculated that maintenance of DNA methylation is incomplete resulting in a gradual decrease of DNA methylation during cell proliferation that should occur equally in all cells but data derived from MSC are contradictory. An alternative possibility is that the types of cell present in what we define as an MSC population change with age so that DNA methylation differences may simply reflect the possibility that different cells are present. Set against this, the analysis made by Horvath [26] included MSC derived from a range of donor ages and show a significant correlation between age and the levels of DNA methylation at the specific CpG islands identified in the study. The DNA methylation levels were partially reset by generating iPSC from such MSC populations but once again this raises the possibility that iPSC clones may have arisen from cells that had somehow escaped the accumulation of epigenetic (or genetic) errors as we observed for the data of Wahlestedt et al.

Can the Microenvironment Change Stem Cell Characteristics?

An interesting addition to the data supporting a significant epigenetic contribution to stem cell ageing comes from the apparent ability of some adult stem cells to reacquire a youthful ability to regenerate their tissue compartments through changes in their microenvironment. Repair/regeneration of the skeletal muscles is most probably performed by satellite cells, a stem cell population residing beneath the basal lamina of the myofibers [80]. Muscle damage results in an inflammatory response causing the normally quiescent satellite cells to proliferate and migrate to the site of damage. Substantial numbers of their progeny undergo differentiation into terminally differentiated myocytes which exit the cell cycle and fuse with existing myofibers to effect their repair. The age-related decline in muscle mass and strength known as sarcopenia is thought to result, at least in part from compromised satellite cell function [81]. Studies of C2C12 cells (a myogenic cell line similar to satellite cells) suggest that levels of DNA methylation increase at the loci of key genes in muscle regeneration such as myogenin [82] with increasing numbers of population doublings. This effect is reversible by administration of 5-azacytidine but a more interesting demonstration of functional rejuvenation comes from heterochronic parabiosis experiments in which the circulatory system of old and young animals was surgically joined [83, 84]. Satellite cells isolated from old humans and mice show similar functional defects in their NOTCH and TGFβ signaling pathways but factors present in the peripheral blood of young mice are able to restore these to a state of activity similar to those of young mouse satellite cells. These data imply that the age-related decline in satellite cell function may not arise because of irreversible errors such as the accumulation of DNA damage but may be due to reversible epigenetic changes. Moreover, the satellite cell population shows little evidence of DNA damage accumulation in older animals. Enumeration of DSBs by detection of γH2A.X foci showed no differences between satellite cells of 4-month-old mice when compared with 24-month-old animals [85]. In addition, SCID mice, which have well-characterized defects in DNA repair, have similar myogenic characteristics to non-SCID mice supporting the idea that DNA repair defects or accumulation of unrepaired DNA damage does not influence satellite cell function when the damage is restricted to the satellite cells. An alternative explanation for the effects of microenvironment on stem cell function could be the senescence-associated secretory phenotype [86, 87] in which the progressive accumulation of senescent cells within a tissue alters the microenvironment by secreting various factors such as interleukin-6. The regulation of senescent cell accumulation and impact upon the progression of an aged phenotype are not clear but published data suggest that that removal of senescent cells can prevent or delay tissue dysfunction [88]. For these reasons, studying satellite cell function in mice derived from iPSC generated from truly aged HSC as proposed above will be a valuable and timely undertaking.

The rejuvenating effects of a young systemic environment do not appear to be restricted to the skeletal muscles. Neurogenesis is subject to an age-related decline in the murine central nervous system [89–91] but this seems to be partially rescued in heterochronic parabionts [92]. Again, the decline in neurogenesis seems to be reversible by other factors since deletion of the Wnt antagonist Dkk1 (whose expression increases during normal ageing of the CNS) [93] can increase the self renewal of neural stem cells and contribute to improved spatial learning and memory in older mice, while increases in the level of the cytokine CCL11 in the blood of young mice are sufficient to reduce neurogenesis and CNS function. These observations appear to support an epigenetic contribution to ageing but at present there are insufficient data from which to draw clearer conclusions.

Summary

We have attempted to review the current evidence supporting a contribution of age-related epigenetic changes to the overall aged phenotype of adult, tissue-specific stem cells. We do not intend to suggest that epigenetic change is the only causative factor in ageing for there can be little doubt that all

mitotically active cell types, even relatively quiescent stem cells, must accumulate some mutations as a consequence of DNA replication. Similarly, the accumulation of unrepaired errors in the less efficiently repaired genomes of the mitochondria must occur in all if not all cell types to some degree. The questions that should now be asked concern the relative contributions of these three possible mechanisms leading to cellular dysfunction. The derivation of induced pluripotent stem cells requires sufficient reprogramming of the epigenome to establish a phenotype consistent with one of the earliest stages of embryonic development. Telomeres are regenerated and the fact that mice can be generated that contain tissues derived from iPSC suggests that such cell lines can be a useful means for addressing the relative contributions of increasing mutational load if age-related epigenetic changes have been removed during the reprogramming steps.

Author Contributions

L.A., J.A.-A., M.S., and M.L.: preparation and critical reading of manuscript.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

References

1 Raveh-Amit H, Berzsenyi S, Vas V, et al. Tissue resident stem cells: Till death do us part. Biogerontology 2013;14:573–590.
2 Carlson ME, Conboy IM. Loss of stem cell regenerative capacity within aged niches. Aging Cell 2007;6:371–382.
3 Sager R. Senescence as a mode of tumor suppression. Environ Health Perspect 1991;93:59–62.
4 Weinstein BS, Ciszek D. The reserve-capacity hypothesis: Evolutionary origins and modern implications of the trade-off between tumor-suppression and tissue-repair. Exp Gerontol 2002;37:615–627.
5 Pearson BJ, Sánchez Alvarado A. Regeneration, stem cells, and the evolution of tumor suppression. Cold Spring Harb Symp Quant Biol 2008;73:565–572.
6 Nižetić D, Groet J. Tumorigenesis in Down's syndrome: Big lessons from a small chromosome. Nat Rev Cancer 2012;12:721–732.
7 Garinis GA, van der Horst GT, Vijg J, et al. DNA damage and ageing: New-age ideas for an age-old problem. Nat Cell Biol 2008;10:1241–1247.
8 Lombard DB, Chua KF, Mostoslavsky R, et al. DNA repair, genome stability, and aging. Cell 2005;120:497–512.
9 Vijg J. Impact of genome instability on transcription regulation of aging and senescence. Mech Ageing Dev 2004;125:747–753.
10 Campisi J. Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. Cell 2005;120:513–522.
11 Saretzki G, Von Zglinicki T. Replicative aging, telomeres, and oxidative stress. Ann N Y Acad Sci 2002;959:24–29.
12 von Zglinicki T, Petrie J, Kirkwood TB. Telomere-driven replicative senescence is a stress response. Nat Biotechnol 2003;21:229–230.
13 Bohr VA. Rising from the RecQ-age: The role of human RecQ helicases in genome maintenance. Trends Biochem Sci 2008;33:609–620.
14 Gray MD, Shen JC, Kamath-Loeb AS, et al. The Werner syndrome protein is a DNA helicase. Nat Genet 1997;17:100–103.
15 De Sandre-Giovannoli A, Bernard R, Cau P, et al. Lamin a truncation in Hutchinson-Gilford progeria. Science 2003;300:2055.
16 Vilchez D, Simic MS, Dillin A. Proteostasis and aging of stem cells. Trends Cell Biol 2014;24:161–170.
17 Arslan MA, Csermely P, Soti C. Protein homeostasis and molecular chaperones in aging. Biogerontology 2006;7:383–389.
18 Porta EA. Pigments in aging: An overview. Ann N Y Acad Sci 2002;959:57–65.
19 Jung T, Bader N, Grune T. Lipofuscin: Formation, distribution, and metabolic consequences. Ann N Y Acad Sci 2007;1119:97–111.
20 Chambers SM, Shaw CA, Gatza C, et al. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS Biol 2007;5:e201.
21 Warren LA, Rossi DJ. Stem cells and aging in the hematopoietic system. Mech Ageing Dev 2009;130:46–53.
22 Sinclair DA, Oberdoerffer P. The ageing epigenome: Damaged beyond repair? Ageing Res Rev 2009;8:189–198.
23 Di Bernardo G, Cipollaro M, Galderisi U. Chromatin modification and senescence. Curr Pharm Des 2012;18:1686–1693.
24 Maegawa S, Hinkal G, Kim HS, et al. Widespread and tissue specific age-related DNA methylation changes in mice. Genome Res 2010;20:332–340.
25 Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell 2013;49:359–367.
26 Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013;14:R115.
27 Jenuwein T, Allis CD. Translating the histone code. Science 2001;293:1074–1080.
28 Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000;403:41–45.
29 Dimauro T, David G. Chromatin modifications: The driving force of senescence and aging? Aging (Albany NY) 2009;1:182–190.
30 Shin DM, Kucia M, Ratajczak MZ. Nuclear and chromatin reorganization during cell senescence and aging—A mini-review. Gerontology 2011;57:76–84.
31 Rhie BH, Song YH, Ryu HY, et al. Cellular aging is associated with increased ubiquitylation of histone H2B in yeast telomeric heterochromatin. Biochem Biophys Res Commun 2013;439:570–575.
32 Liu L, Cheung TH, Charville GW, et al. Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. Cell Rep 2013;4:189–204.
33 Sarraf SA, Stancheva I. Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. Mol Cell 2004;15:595–605.
34 Davey CS, Pennings S, Reilly C, et al. A determining influence for CpG dinucleotides on nucleosome positioning in vitro. Nucleic Acids Res 2004;32:4322–4331.
35 Fuks F. DNA methylation and histone modifications: Teaming up to silence genes. Curr Opin Genet Dev 2005;15:490–495.
36 Harman D. The aging process. Proc Natl Acad Sci USA 1981;78:7124–7128.
37 Harman D. The free radical theory of aging. Antioxid Redox Signal 2003;5:557–561.
38 Croteau DL, Bohr VA. Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells. J Biol Chem 1997;272:25409–25412.
39 Khrapko K, Vijg J. Mitochondrial DNA mutations and aging: Devils in the details? Trends Genet 2009;25:91–98.
40 Greaves LC, Barron MJ, Plusa S, et al. Defects in multiple complexes of the respiratory chain are present in ageing human colonic crypts. Exp Gerontol 2010;45:573–579.
41 Pang WW, Price EA, Sahoo D, et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci USA 2011;108:20012–20017.
42 Kuranda K, Vargaftig J, de la Rochere P, et al. Age-related changes in human hematopoietic stem/progenitor cells. Aging Cell 2011;10:542–546.
43 Muller-Sieburg C, Sieburg HB. Stem cell aging: Survival of the laziest? Cell Cycle 2008;7:3798–3804.
44 Dykstra B, Olthof S, Schreuder J, et al. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. J Exp Med 2011;208:2691–2703.
45 Beerman I, Bhattacharya D, Zandi S, et al. Functionally distinct hematopoietic

stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. Proc Natl Acad Sci USA 2010;107:5465–5470.
46 Solbach U, Keilhauer C, Knabben H, et al. Imaging of retinal autofluorescence in patients with age-related macular degeneration. Retina 1997;17:385–389.
47 Fuchs R, Schraml E, Leitinger G, et al. α1-Adrenergic drugs modulate differentiation and cell death of human erythroleukemia cells through non adrenergic mechanism. Exp Cell Res 2011;317:2239–2251.
48 Yahata T, Takanashi T, Muguruma Y, et al. Accumulation of oxidative DNA damage restricts the self-renewal capacity of human hematopoietic stem cells. Blood 2011;118: 2941–2950.
49 Shin MG, Kajigaya S, McCoy JP Jr, et al. Marked mitochondrial DNA sequence heterogeneity in single CD34+ cell clones from normal adult bone marrow. Blood 2004;103: 553–561.
50 Yao YG, Kajigaya S, Feng X, et al. Accumulation of mtDNA variations in human single CD34+ cells from maternally related individuals: Effects of aging and family genetic background. Stem Cell Res 2013;10: 361–370.
51 Norddahl GL, Pronk CJ, Wahlestedt M, et al. Accumulating mitochondrial DNA mutations drive premature hematopoietic aging phenotypes distinct from physiological stem cell aging. Cell Stem Cell 2011;8:499–510.
52 Florian MC, Geiger H. Concise review: Polarity in stem cells, disease, and aging. Stem Cells 2010;8:1623–1629.
53 Florian MC, Dörr K, Niebel A, et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell Stem Cell 2012;10:520–530.
54 Rios RM, Bornens M. The Golgi apparatus at the cell centre. Curr Opin Cell Biol 2003;15:60–66.
55 Krummel MF, Macara I. Maintenance and modulation of T cell polarity. Nat Immunol 2006;7:1143–1149.
56 Geiger H, Koehler A, Gunzer M. Stem cells, aging, niche, adhesion and Cdc42: A model for changes in cell-cell interactions and hematopoietic stem cell aging. Cell Cycle 2007;6:884–887.
57 Xing Z, Ryan MA, Daria D, et al. Increased hematopoietic stem cell mobilization in aged mice. Blood 2006;108:2190–2197.
58 Wang L, Yang L, Debidda M, et al. Cdc42 GTPase-activating protein deficiency promotes genomic instability and premature aging-like phenotypes. Proc Natl Acad Sci USA 2007;104:1248–1253.
59 Apostolou E, Hochedlinger K. Chromatin dynamics during cellular reprogramming. Nature 2013;502:462–471.
60 Wahlestedt M, Norddahl GL, Sten G, et al. An epigenetic component of hematopoietic stem cell aging amenable to reprogramming into a young state. Blood 2013;121: 4257–4264.
61 Chamberlain G, Fox J, Ashton B, et al. Concise review: Mesenchymal stem cells: Their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007;25:2739–2749.
62 Nöth U, Rackwitz L, Steinert AF, et al. Cell delivery therapeutics for musculoskeletal regeneration. Adv Drug Deliv Rev 2010;62: 765–783.
63 Chen Y, Shao JZ, Xiang LX, et al. Mesenchymal stem cells: A promising candidate in regenerative medicine. Int J Biochem Cell Biol 2008;40:815–820.
64 Wu XB, Tao R. Hepatocyte differentiation of mesenchymal stem cells. Hepatobiliary Pancreat Dis Int 2012;11:360–371.
65 Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284: 143–147.
66 Lepperdinger G. Inflammation and mesenchymal stem cell aging. Curr Opin Immunol 2011;23:518–524.
67 Chen JH, Yip CY, Sone ED, et al. Identification and characterization of aortic valve mesenchymal progenitor cells with robust osteogenic calcification potential. Am J Pathol 2009;174:1109–1119.
68 Choumerianou DM, Martimianaki G, Stiakaki E, et al. Comparative study of stemness characteristics of mesenchymal cells from bone marrow of children and adults. Cytotherapy 2010;12:881–887.
69 Wagner W, Bork S, Horn P, et al. Aging and replicative senescence have related effects on human stem and progenitor cells. PLoS One 2009;4:e5846.
70 Laschober GT, Brunauer R, Jamnig A, et al. Age-specific changes of mesenchymal stem cells are paralleled by upregulation of CD106 expression as a response to an inflammatory environment. Rejuvenat Res 2011;14:119–131.
71 Zhang J, Lian Q, Zhu G, et al. A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects. Cell Stem Cell 2011;8:31–45.
72 Csoka AB, English SB, Simkevich CP, et al. Genome-scale expression profiling of Hutchinson-Gilford progeria syndrome reveals widespread transcriptional misregulation leading to mesodermal/mesenchymal defects and accelerated atherosclerosis. Aging Cell 2004;3:235–243.
73 Scaffidi P, Misteli T. Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. Nat Cell Biol 2008; 10:452–459.
74 Cao K, Blair CD, Faddah DA, et al. Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. J Clin Invest 2011;121: 2833–2844.
75 Miller JD, Ganat YM, Kishinevsky S, et al. Human iPSC-based modeling of late-onset disease via progerin-induced aging. Cell Stem Cell 2013;13:691–705.
76 Alves H, Munoz-Najar U, De Wit J, et al. A link between the accumulation of DNA damage and loss of multi-potency of human mesenchymal stromal cells. J Cell Mol Med 2010;14:2729–2738.
77 Altanerova V, Horvathova E, Matuskova M, et al. Genotoxic damage of human adipose-tissue derived mesenchymal stem cells triggers their terminal differentiation. Neoplasma 2009;56:542–547.
78 Galderisi U, Helmbold H, Squillaro T, et al. In vitro senescence of rat mesenchymal stem cells is accompanied by downregulation of stemness-related and DNA damage repair genes. Stem Cells Dev 2009;18:1033–1042.
79 Bork S, Pfister S, Witt H, et al. DNA methylation pattern changes upon long-term culture and aging of human mesenchymal stromal cells. Aging Cell 2010;9:54–63.
80 Saini A, Mastana S, Myers F, et al. 'From death, lead me to immortality'—Mantra of ageing skeletal muscle. Curr Genomics 2013; 14:256–267.
81 Zammit PS, Partridge TA, Yablonka-Reuveni Z. The skeletal muscle satellite cell: The stem cell that came in from the cold. J Histochem Cytochem 2006;54:1177–1191.
82 Sharples AP, Al-Shanti N, Lewis MP, et al. Reduction of myoblast differentiation following multiple population doublings in mouse C2 C12 cells: A model to investigate ageing? J Cell Biochem 2011;112:3773–3785.
83 Conboy IM, Rando TA. Aging, stem cells and tissue regeneration: Lessons from muscle. Cell Cycle 2005;4:407–410.
84 Brack AS, Conboy MJ, Roy S, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 2007;317:807–810.
85 Cousin W, Ho ML, Desai R, et al. Regenerative capacity of old muscle stem cells declines without significant accumulation of DNA damage. PLoS One 2013;8:e63528.
86 Rodier F, Coppé JP, Patil CK, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol 2009;11:973–979.
87 Tchkonia T, Zhu Y, van Deursen J, et al. Cellular senescence and the senescent secretory phenotype: Therapeutic opportunities. J Clin Invest 2013;123:966–972.
88 Baker DJ, Wijshake T, Tchkonia T, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 2011;479:232–236.
89 Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation. J Neurosci 1996;16: 2027–2033.
90 Maslov AY, Barone TA, Plunkett RJ, et al. Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. J Neurosci 2004;24:1726–1733.
91 Molofsky AV, Slutsky SG, Joseph NM, et al. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. Nature 2006;443:448–452.
92 Villeda SA, Luo J, Mosher KI, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 2011;477:90–94.
93 Seib DR, Corsini NS, Ellwanger K, et al. Loss of Dickkopf-1 restores neurogenesis in old age and counteracts cognitive decline. Cell Stem Cell 2013;12:204–214.

