Metabolic Control of Stem Cell Ageing and Longevity through Caloric Restriction

By Valerie Navarrete

Department of Molecular, Cellular and Developmental Biology, Yale University; Department of Art, Yale University

ABSTRACT

While prior studies have identified recurring genetic patterns, gaps of knowledge still remain in existing aging mechanisms; where they originate, and how they offer insight to environmental disruptions that dictate health over time. Given the inescapability of age-related deterioration and pathology, stitching together current literature may help demystify the biological process common to all living mammals. The physiological disruption of aged tissue reflects a cellular dependence on environmental cues and historical wear. Retaining the capacity to differentiate into any cell type, a stem cell best parallels a call-and-response relationship between organ and cell. As the longest living proliferative cell in multicellular organisms, stem cells respond to environmental cues through genomic or proteomic shifts. Aging tends to disrupt this capability, as stem cells lose functionality over time. This review will focus on the mechanisms associated with stem cell depletion and skin tissue degeneration. By concentrating on genetic pathways common in studies comparing caloric restriction models in young and old species, this review will highlight commonalities that generate age-related stem cell depletion and tissue degeneration.

CALORIC RESTRICTION (CR) AS AN ANTI-AGING INFLUENCER

In 2019, the 65+ year old age group represented 16.5 percent of the American population. By 2050, this number is expected to reach 22 percent, roughly one quarter of the entire nation. While improved livelihood is a signifier of a developed nation’s technological, scientific, and medical advancements, the degenerative repercussions of aging are hardly understood. The onset of chronic illness can burden hefty economic costs from nursing homes, medical treatment, and health insurance. In addition to monetary expenses, age is correlated with high risk of developing chronic disease. Unfortunately, aging is not a manifestation of one particular molecular disturbance, but results from a series of microscopic alterations, that culminate in external and internal deterioration. In other words, these changes are not merely triggered by an on switch in the body, but a history of environmental and innate interactions.

Witnessing phenotypic indications like decelerated mobility, stark facial changes, and body mass alterations, scientists have been able to name aging without necessarily understanding it. In a review of modern aging research, Professor Kayvan Zainbadi stresses that all living beings have a common denominator named death; and it is the rate of reaching that point which motivates modern aging studies (Zainbadi, 2018). With the eventual aid of scientific advancements in genomics, researchers were finally able to unveil precise biomarkers such as: IL-6, albumin, leptin, HDL, and IGF-1 (Crimmins, Vasunilashorn, Kim, & Alley, 2008). While these represented mealy pieces in the grand scheme, major mysteries within the aging process were just beginning to unravel.

The first 1935 study was not just influential for its phenotypic characterization of aging itself, but for the model in which it recreated the aged phenotype. Rather than relying on a natural progression over time, researchers elicited the aged phenotype through metabolic manipulation. By manually altering the cellular environment of the organism, caloric restriction (CR) proved a successful, non-invasive model to delay ageing and subdue pathological disease (López-Lluch & Navas, 2016). By reducing energy intake without provoking malnutrition, the aged phenotype was reversed and pathological deterioration was avoided. Not long after this discovery, the National Institute of Aging began extending lifespan 3-fold with CR alone, and performed targeted drug treatment on mice, flies, worms, and yeast, to effectively stretch lifespan up by 10-fold in yeast (Fontana, Partridge, & Longo, 2010). Hispkkiss clarifies this phenomenon in the “Hormesis hypothesis of CR,” which explains how the mild survival pressure triggered by fuel depletion, causes an upsurge in organ and cell protection amidst environmental adversity (Hispkkiss, 2007). Essentially, ensuing pathways of CR activate cell longevity and protection, producing an antiaging phenotype reflective of younger organisms. The main pathway influenced by dietary intervention is the insulin/insulin-like growth fac
tor (IIS) pathway (Santos, Leitão-Correia, Sousa, & Leão, 2016). Many of the IIS pathway constituents have been dissected and analyzed in overlapping studies of CR-induced and aged organisms. Within this overlap, current studies have defined key molecular players responsible for age-related deterioration. Due to the vast stretch of age-related pathology, this review will narrow the scope of aging to a system depicting both of these manifestations.

DETERIORATION OF SKIN EPITHELIUM IN AGING

At the interface of environment and internal anatomy, skin is a unique organ that presents both biological and external repercussions of health detriment. The adult skin epithelium is comprised of a pilosebaceous unit containing a hair follicle (HF) and sebaceous gland. With the need to efficiently respond to environmental cues and damage, homeostatic regulation is crucial in maintaining the integrity of the skin structure and health. In fact, injury to this protective layer increases susceptibility to diseases caused by environmental irritants, and benign or more threatening pathological manifestations like pruritus, carcinomas, or melanomas (Farage, Miller, Elsner, & Maibach, 2013). From an external perspective, most histological studies have reported signs of atrophy, epidermal thinning, and abnormal fiber reconstruction (Bhattacharyya T.K., 2010). Studies specific to mouse-models verify a consistent thinning of the epidermal layer, with shortened and sparse hair follicles in older mice (Bhattacharyya T.K., 2010). Additional studies report atrophied sebaceous glands and decreased dermal cellularity, further suggesting the role of aging in homeostatic cell proliferation and death (Giangreco A, 2008).

CHARACTERISTICS OF A STEM CELL

From a common tissue origin, stem cells self-renew and differentiate into multiple lineages (Blanpain & Fuchs, 2006). The skin possesses different subpopulations of stem cells; each play a role in sustaining organs by differentiating into specialized cell types. Layers within the epidermis encapsulate hair follicles (HFs), sebaceous gland, mammary glands, and sweat (Ge et al., 2020). The hair follicle stem cell (HFSC) sits in a stem cell niche called the bulge—its master regulator. Harboring control over stem cell homeostasis through extracellular communication, the niche contains cells in contact with stem cells and other soluble factors (Gianluigi Mazzoccoli, Tevy, Borghesan, Vergini, & Vinciguerra, 2014). The surrounding dermal papilla, lymphatic capillaries, adipose tissue, and dermal fibroblasts, signal the HFSC to rest (quiescence) or regenerate (differentiation). Rather than self-renewing the HF pool, damage to HFSC DNA causes HFSCs to escape the niche and differentiate to keratinocytes that eventually flake off the skin surface (Matsumura et al., 2016). This mechanism is likely responsible for the hair-thinning phenotype common in aging; elucidating a relationship between hair and skin, activated from damaged DNA. Studies utilizing CR to reverse aged phenotypes discover similar HFSC pool remodeling, in which an increase in quantity and regrowth rate present a decrease in stem cell quantity, with much shorter and sparser fur coats as a result of HF miniaturization (Forni et al., 2017).

This research, alongside prior studies on stem cell regulation and homeostasis, define the roles of nutrient-sensing pathways and metabolic regulation that define the “stem-ness” of stem cells. While the mechanisms of nutrient sensing may differ across varying stem cell species, the quiescence and differentiation switches inherent in both pluripotent and multipotent stem cells, appear to be affected by metabolic cycles such as glycolysis and the IIS pathway (Ochocki & Simon, 2013). Through diet manipulation from the CR condition, there is significant evidence to support the role of nutrient sensing in prompting cues within the stem cell niche, and triggering downstream feedback loops.

HOMEOSTATIC ISSUES IN AGING

By reducing major energy pathways, CR promotes stem cell self-renewal and regeneration, while decreasing differentiation. Stem cell quiescence is necessary for stem cells to pause, and avoid a build-up of epigenetic alterations that compromise function (Ermolaeva, Neri, Ori, & Rudolph, 2018). Exhaustion of stem cell activity can spike an increase of SC proliferation, also initiating cell damage that impairs function and accumulates misfolded proteins (Ermolaeva et al., 2018). Many of the factors that determine this activity are transpired by communication through the stem cell niche. The pathological alterations made to them can be categorized as extrinsic (stem cell niche alterations, systemic factors) or intrinsic (epigenetic changes, DNA damage) (Maharajan, Vijayakumar, Jang, & Cho, 2020).

This explains how the “starvation” mode of CR condition activates a series of proteins involved in autophagy, stem cell self-renewal, and antioxidative activity. Subsequent hormonal shifts in insulin, IGF-1, and leptin, all interplay to inhibit mTOR signaling pathways and downstream effects (Gianluigi Mazzoccoli et al., 2014). In comparison to young and old mice, mTORC1 was expressed more abundantly in old mice overall (Chen, Liu, Liu, & Zheng, 2009). Within the scope of skin, inhibited mTORC1 was found to decrease differentiated epidermal stem cell senescence; which may have been due to a decrease in cytokines circulating around the stem cell niche (Chung et al., 2019). Due to a resulting nutrient abundance, insulin and growth factors increase, autophagy is inhibited, and protein synthesis is encouraged in ad libitum treatments. Additional studies utilizing mTOR have recreated these CR mimetic outcomes, prompting mTOR as a pivotal gene in stem cell communication.

Achiving longevity while compensating fuel loss is essential in reproducing the anti-aging effects of CR. In response to low food intake, autophagy pathways recycle unwanted organelles in the body, and refuel the cell for upcoming biosynthetic reactions (G. Mazzoccoli, Tevy, Borghesan, Delle Vergini, & Vinciguerra, 2014). When exogenous substrates are absent, lysosomes degrade unwanted cargo, and autophagy salvages the debris to produce a source of amino acids for glucogenesis. CR induces autophagy upon this same “starvation” mechanism. AMP-activated protein kinase (AMPK) is one of the factors that senses nutritional changes during CR, inhibits expression of mTORC1, and switches on catabolic pathways to produce ATP (Cantó et al., 2009). This amplified metabolic activity
increases NAD+ in the cell environment, inadvertently triggering SIRT1—a factor responsible for deacetylating FOXO1 (Pan & Finkel, 2017). In order to better understand this responsive mechanism transpired by the IIS pathway, the individual responsibilities for each gene should be closely examined; alongside comparative studies that elicit similar responses amongst young and CR-treated organisms.

**FOXO TRANSCRIPTION FACTORS**

The FOXO family of transcription factors have demonstrated pro-longevity effects in prior ageing studies unrelated to stem cell function. They often serve as a major substrate of protein kinase Akt in the presence of growth factors or insulin (Greer & Brunet, 2005). When found in the nucleus, they upregulate a series of target genes responsible for stress resistance, cell cycle arrest, or apoptosis (Greer & Brunet, 2005). However, they only remain in the nucleus in the absence of growth factors and insulin (Greer & Brunet, 2005).

Subcellular localization of FOXO from environmental cues such as growth factors and insulin, determines whether or not FOXO interacts with cell regulatory gene expression (Hosaka et al., 2004). In the presence of factors such as insulin or growth factor (GF), FOXO proteins localize to the cytoplasm (Hosaka et al., 2004). This sequestration promotes expression of cell proliferation and stress sensitivity. The resulting metabolic shift from glucose to lipid oxidation suppresses inflammation and induces mitochondrial biogenesis (van Heemst, 2010). With a heavy influence of FOXO activation upon cell-cycle and replication, these transcription factors are often involved in cancer studies focused on tumor suppression and organismal longevity (van Heemst, 2010).

In addition to its involvement in epidermal stem cells (epSCs), FOXO proteins also prep Hematopoietic stem cells (HSCs) for autophagy during CR (Hosaka et al., 2004). FOXO upregulation in mice HSCs induce vigorous autophagy under the CR condition, and markedly downregulate pro-autophagic targets upon deletion (Warr et al., 2013). Not only does this upregulation in HSCs suggest a common function of FOXO across various stem cells, but CR’s influence on HSCs can also play a role in shifting the niche environment of epSCs, through modulation of reactive oxygen species (Ludin et al., 2014). Additional to CR studies, many studies have explored differences in these nutritional profiles of niches between young and old mice. The presence of 2-NBD glucose was the single identified difference between young and old mice with different autophagy fluxes (Warr et al., 2013). Preparing an equipped responsiveness in SCs, FOXO proteins represent a necessary and conserved effort to alleviate strains of an energy crisis (Warr et al., 2013). Between wild type and SIRT1 knockout samples from mice, SIRT1 knockouts present debilitated self-renewing capacity, suggesting SIRT1 pivotal in maintaining a stem cell’s “stemness” (Matsui et al., 2012).

Sirtuins have been described as nicotinamide adenine dinucleotide (NAD)-dependent protein type III histone deacetylases (HDACs) (Schemies, Uciechowska, Sippl, & Jung, 2010). This class of proteins deacetylates histones on DNA, allowing room to bind DNA around histones of nucleosomes (Schemies, 2010). This is a clever epigenetic mechanism, which allows sirtuins to influence expression of genes involved in DNA damage, stress response, and lipid metabolism (Ermolaeva, M. & Rudolph, K. L., 2018). As implied by the name, this class of protein also requires an NAD+ substrate; and the presence of such is highly determined by the nutritional status of the cell. Thus, as the CR condition triggers a shift in the nutritional environment of the SC, the sirtuin protein—and its downstream effects—are activated as a result (López-Lluch, G., & Navas, P., 2016). Sirtuins also silence recombination between recombinant DNA (rDNA) repeats, while mutation of Sir2 results in a marked accumulation of extrachromosomal rDNA circles (ERCs) that induce nuclear fragmentation, cessation of cell division, and cellular senescence (Sinclair & Guarente, 1997).

**SIRTUIN NAD-DEPENDENT PROTEINS**

Downstream to the IGF-1 signaling pathways, SIRT1 is activated under CR conditions in which growth factors and insulin are sparse (Cohen et al., 2004). Through ROS elimination, sirtuins encourage stem cell self-renewal, function and regeneration (Matsui et al., 2012). One of its main targets is the FOXO transcription factor, which as previously discussed, plays a major role in SC autophagy. Between wild type and SIRT1 knockout samples from mice, SIRT1 knockouts present debilitated self-renewing capacity, suggesting SIRT1 pivotal in maintaining a stem cell’s “stemness” (Matsui et al., 2012).

**Figure 1** (created through BioRender, 2020). Broad gene schematic of various pathways influenced by CR. Insulin and IGF-1 normally trigger the IIS pathway, activating Akt—a known inhibitor of FOXO. Lack of insulin and IGF-1 through CR results in activation of FOXO. Additional activation of FOXO is activated by deacetylation from SIRT1, which is also activated by nutritional loss from the CR condition. mTOR is regulated by inhibition of AMPK, and activation by nutritional loss.
EPIGENETIC REPROGRAMMING

Proliferation of epSCs follow a rhythmic regulation of activation and quiescent pathways. In order to circumvent genotoxic lesions caused by maximum oxidative phosphorylation in the day, mice epSCs replicate their DNA in the night (Solanas et al., 2017). While this cycle may be expected to diverge over time, aged epSCs remain just as rhythmic as their younger counterparts (Solanas et al., 2017). That being said, their epSCs are still forced to rewire and adapt to environmental challenges that would maximize energy efficiency (Solanas et al., 2017). Initially, it was presumed that genetic alterations were responsible for this inefficient activation (Solanas et al., 2017). However, upon knocking out genetic controllers identified with the circadian rhythm, the aged phenotype was not recreated; suggesting some other type of genetic remodeling to have produced these effects in young and old mice (Solanas et al., 2017). While this study did not pursue epigenetic analysis further, the inability to reproduce this same rewiring with knockouts, may suggest epigenetic alterations as responsible for inefficient energy usage over time.

Epigenetic reprogramming in SCs has prompted interest for its influence on homeostatic regulation. The SC genome can be altered easily by environmental stimuli, which determines expression of epigenetic regulators. Relaying inflammatory cues such as cytokines, growth factors, or adhesions molecules, the stem cell niche may have a strong influence on compounding aged phenotypes. If certain cues regulate gene expression, new shifts in SC activity can inadvertently influence tissue health and homeostasis. For example, DNA methylation has been found to suppress genes related to cell cycle exit and keratinocyte differentiation from epSCs; which can lead to possible atrophying of the epidermal surface (Mulder et al., 2012).

CLONAL EXPANSION

Amidst challenges to maintain SC homeostasis, epigenetic alterations in certain daughter cells can ripple subsequent mutations in SC progeny. Expansion across multiple progenies create a clone dominance that outweighs the normal SC gene profile and impairs tissue homeostasis. This process is defined as epigenetic drift and clonal expansion, and is characterized by age-dependent changes in the CpG methylome, heterochromatinization of precise areas of the genome, and reinforcement of open chromatin regions (Kirschner et al., 2017). Impairments in key epigenetic modifiers through this epigenetic drift have been found to mutate HSCs, and incite damage in cell differentiation, tissue dysfunction, and cancers (Kirschner et al., 2017).

Transition from stem cell to transient amplifying cells defines the clonal expansion process. Clonal expansion of epSCs is highly regulated by tumor suppressors such as p16INK4a. By inhibiting the G1/S-phase transition in cell cycle, P16INK4a governs SC self-renewal in skin tissue (Orioli & Dellambra, 2018). However, as an aberrant chromatin sensor, p16INK4a induces tumor suppressive cell senescence pathways, which can exhaust epithelial stem cells over time (Iglesias-Bartolome, Callejas-Valera, & Gutkind, 2013). Thus, maintenance of skin SC population homeostasis is highly dependent on p16INK4a repression, as it directly coordinates a...
genetic balance between keratinocyte renewal and differentiation (D’Arcangelo, Tinaburri, & Dellambara, 2017). Multiple epigenetic alterations can target the repression of P16INK4a, including the usage of histone deacetylases (HDAC) (D’Arcangelo et al., 2017). SIRT1 actually serves as an HDAC under CR. The scarcity of free-floating glucose under CR, actually causes SIRT1 to suppress P16INK4a through direct deacetylation, just as those that balance keratinocyte renewal and differentiation (Li & Tollefsbol, 2011).

**STEPS FORWARD IN PHARMACEUTICAL APPROACHES AND FUTURE STUDY**

Regarding stem cell longevity, anti-aging therapeutics should strike a balance between SC senescence and quiescence. We’ve reviewed how the IIS pathway altered by CR can influence either fate, by initiating specific nutritional shifts and epigenetic alterations. That being said, genetic control of homeostasis must be approached with caution. A pathological imbalance can tip SCs to extreme self-renewal or differentiation (Oh et al., 2014).

In order to gain control of this process, countless studies have suggested bloodborne factors as an indirect focus for stem cell engineering. GDF11 supplementation was found to reverse hypertrophy in cardiac muscle through activation of TGF-beta pathways in human-induced pluripotent stem cell-derived cardiomyocytes (Loffredo et al., 2013). Utilizing some of the key players addressed in CR studies, direct inhibition of mTORC1 through dTsc1 suppression in c. elegans and drosophila, were found to extend lifespan as well (Jia, Chen, & Riddle, 2004) (Kapahi et al., 2004). Pharmaceutical inhibition of mTOR by rapamycin alone, redeemed self-renewal and hematopoietic potential in mice (Chen et al., 2009).

Metformin, an existing Diabetes treatment that enhances insulin sensitivity, is another drug that targets downstream players of the IIS pathway. Since AMP-activated protein kinase activity is a direct target of this drug, the effects on physical performance, insulin sensitivity, and reduced cholesterol levels, all mimic those of the CR condition in mice (Martin-Montalvo et al., 2013). Chronic exposure to metformin in mice lengthened lifespan and produced anti-aging properties (Martin-Montalvo et al., 2013). However, the dosage associated with mice was much higher than that typically used in diabetes treatment (Martin-Montalvo et al., 2013). Because the pharmacokinetic disruptions from chronic exposure levels are not fully understood, more studies are necessary to determine the efficacy and safety in humans.

Alongside systemic regulators, direct targeting of senescent cells and their products in aged tissue, is another therapeutic possibility. One study designed a drug containing a transgene that eliminated P16INK4a-positive senescent cells (Baker et al., 2011). The adipose tissue of mice that received life-long treatment, demonstrated delayed aging phenotypes typical in p16INK4a active cell types (Baker et al., 2011). Furthermore, ablation of p16INK4a in aged mice reverted pathological deterioration in skin tissue (Baker et al., 2011).

Epigenetic modifiers identified by CR, have suggested the possibility of incorporating epigenetic clocks in future anti-aging studies. Epigenetic clocks utilize DNA methylation-based biomarkers to estimate age based on tissue status. Horvath’s and Hannum’s clocks, for example, predict age based on measured DNAm values in specific CpG sites (Horvath, 2013). Epigenetic clocks in ageing studies represent a non-invasive biomarker, and may offer more accurate insight on the health status of humans. In comparison to actual age, epigenetic age is more accurate in defining health status, due to its association with pathology and disease risk. Currently, epigenetic clocks in rodents are developed in blood-based and liver-based clocks (T. Wang et al., 2017). These clocks serve as a measure for aged phenotypes in CR studies, with CR-treated rodents presenting epigenetically younger ages compared to their ad-libtum counterparts (Maegawa et al., 2017; T. Wang et al., 2017). Even in humans, epigenetic clocks have been utilized to define correlations between epigenetic age and environmental factors like diet, exercise, and education status (Quach et al., 2017).

While this review highlighted successful CR studies that produced antiaging properties and stem cell longevity, the translation to humans should be considered more thoughtfully. Despite extreme diet trends that alter calories in hopes of weight loss, prolonged reduction of calories can elicit equally degenerative properties in humans (G. Mazzoccoli et al., 2014). In fact, gerontologists often worry that CR can misguide patients as a “quick fix,” warning that drastic caloric manipulations in fad diets, can easily result in extreme frailty or obesity (Pifferi & Aujard, 2019). The elderly population already faces significant risk from physiological frailty imposed by extreme weight loss. CR should only be a means to study genetic relationships in model organisms—a tool to advance our understanding of aging mechanisms and possible pharmaceutical interventions.

**CONCLUSION**

This review focused on age-related pathologies found in the skin, caused by malfunctioning stem cell homeostasis. We considered CR-related studies to better understand how the IIS pathway relates to anti-aging therapeutics. Through the genetic mechanisms of mTORC1, FOXO1, SIRT1, and AMPK, we initially clarified how environmental factors influence nutritional status in the stem cell niche, and trigger genetic cascades that encourage adaptive response pathways under starvation conditions. The epigenetic alterations embedded in these response pathways, alongside their influence on stem cell function and skin homeostasis, were further investigated in studies both on aged and CR-treated organisms. We concluded with pharmaceutical translations, and how future directions inspired by CR genetic pathways could lead to advancements in therapeutics that address age-related pathologies.

While further studies on CR and its supporting mechanistic routes are necessary to better define “aging,” a strong understanding of pathophysiological features can help address disease risks more effectively. Additionally, the therapeutic advancements inspired by this research withholds possibilities to better define health status, as the definition of good health, is not aptly captured by surface-level metrics. Likewise, the pathway to good health is not paved equally for everyone. While this remains an untapped field, perhaps by
learning from the environmental-biological synergy elucidated in this paper; we can finally allow scientific inquiry to address systemic inequalities, and regard external factors as a source of health detriment.

REFERENCES


longevity, 2016, 4010357-4010357. doi:10.1155/2016/4010357


After a long day at the Horsley lab investigating the role of caloric restriction on mice, Valerie Navarrete ’21 turns to her creative outlet, art, to relax and de-stress. As a double-major student in Molecular, Cellular, and Developmental Biology and art, she ultimately hopes to explore science and medicine from an artistic lens. At the Horsley lab, Navarrete works with her mentor, Valerie Horsley, on the effect of caloric restriction on mice. In her experiments, they discovered phenotypic differences between two groups of mice, where the one with caloric restriction visibly and internally looked younger than the group of mice with unrestricted feeding. To further investigate the mechanism and aging process, she analyzed the role of nutrition and environment on stem cell populations at the cellular level and epigenetic level. Navarrete first became acquainted with Professor Horsley through her class, Biology 102: The Cell. “I always thought she was so cool and cared so much about her students,” she said. Later on, she became interested in stem cell research after reading a newsletter spotlighting Professor Horsley’s research on stem cells her first year. When she started emailing professors for a lab position in her junior year, Navarrete remembered Horsley’s research and decided to reach out. She credits Horsley and her mentors in the lab for solidifying her interest in STEM and biology. “I love her lab. I think her lab, my mentors and all the women that are a part of that collective is a big reason why I feel confident in biology now and why I love science so much. I want to pursue an MD/Ph.D. down the road, but before that, I wasn’t really big on research because of the whole stereotype and stigmas behind it,” Navarrete said. Outside of the Horsley lab, Navarrete illustrates for the Yale Daily News and actively participates in Yale’s Slavic chorus. She also paints and sketches in her free time. “I’m using art to understand myself, my history, my identity. And once I do that I could really get into the meat doing it for other people and finding how to apply it in the scientific realm,” Navarrete said. After graduation she is considering attending a program with Rhode Island School of Design (RISD), in order to gain some artistic and clinical experience before applying to MD/Ph.D programs in the future.

For the full-length profile, visit yalesymposia.com