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The matrisome during aging and longevity: a systems-level approach towards defining matreotypes promoting healthy aging

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Abstract

Accumulation of damage is generally considered the cause of aging. Interventions that delay aging mobilize mechanisms that protect and repair cellular components. Consequently, research has been focused on studying the protective and homeostatic mechanisms within cells. However, in humans and other multicellular organisms, cells are surrounded by extracellular matrices (ECM), which are important for tissue structure, function and intercellular communication. During aging, components of the ECM become damaged through fragmentation, glycation, crosslinking, and accumulation of protein aggregation, all of which contribute to age-related pathologies. Interestingly, placing senescent cells into a young ECM rejuvenates them and we found that many longevity-assurances pathways re-activate *de-novo* synthesis of ECM proteins during aging. This raises the question of what constitutes a young ECM to reverse aging or maintain health? In order to make inroads to answering this question, I suggest a systems-level approach of quantifying the matrisome or ECM compositions reflecting health, pathology, or phenotype and propose a novel term, the "matreotype", to describe this. The matreotype is defined as the composition and modification of ECM or matrisome proteins associated with or caused by a phenotype, such as longevity, or a distinct and acute physiological state, as observed during aging or disease. Every cell type produces its unique ECM. Interestingly, cancer-cell types can even be identified based on their unique ECM composition. Thus, the matreotype reflects cellular identity and physiological status. Defined matreotypes could be used as biomarkers or prognostic factors for disease or health status during aging with potential relevance for personalized medicine. Treatment with biologics that alter ECM-to-cell mechanotransduction might be a strategy to reverse age-associated pathologies. An understanding of how to reverse from an old to a young matreotype might point towards novel strategies to rejuvenate cells and help maintain tissue homeostasis to promote health during aging.

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Introduction

Cells and tissues are embedded within extracellular matrices (ECM), which are important for tissue geometry, integrity, and function [1]. Besides providing structural support, the ECM controls intercellular communication by either storing or transporting signaling molecules, such as growth factors, hormones, and neuropeptides [1-3]. Receptors on the cell surface, such as integrins, directly link ECM to cell signaling to regulate various biological functions (Figure 1). Cells synthesize and secret ECM proteins, such as collagens, extracellular glycoproteins, and proteoglycans that are integrated into matrices by extracellular enzymes [2]. Cells also continuously monitor and integrate signals from the ECM via mechanotransduction and adjust their surrounding environment by synthesizing and remodeling their ECM [2]. Thus, the ECM at least during development and normal tissue homeostasis is a dynamic system.

During aging, the connective tissue or ECM integrity declines through accumulation of damage from collagen fragmentation, oxidation, glycation, crosslinking, and accumulation of protein aggregates (Figure 1), leading to a decline in ECM dynamics and loss of organs support and function [3,4]. This decline in ECM integrity has been implicated in many age-dependent diseases, such as atherosclerosis, diabetes, and cancer. Accumulation of damage to the ECM drives cellular aging and disease progression. Below, I discuss how collagen fragmentation, collagen glycation and crosslinking, and aggregation of proteins in the extracellular space affect ECM integrity to accelerate aging. Moreover, longevity-assurance pathways might slow aging by activating collagen and ECM remodeling to protect from this progressive decline in ECM integrity.

Progressive decline in ECM turnover and dynamics during aging

Protein homeostasis is defined as the cellular regulation of synthesis, folding, trafficking and degradation of proteins. In contrast to unfolded intracellular proteins that can be refolded through chaperones, damaged and unfolded ECM proteins need to be excised out of the matrix and novel ECM proteins must be synthesized to replace them (Figure 1A). Since this turnover requires cells to be adjacent to the ECM, ECM structures that are acellular lack this dynamic turnover. For example, certain collagens in the eye lenses or cartilages of humans are only once synthesized and integrated into the ECM and remain there lifelong with spectacular half-life times of over 117 years [4,5]. On the other hand, collagens in other tissues can be turned over extremely

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fast, for example within 72 hours in the Achilles tendons after exercise [6]. During aging, either through collagen fragmentation or loss of adherence proteins [7], cells detach from the ECM potentially leading to cell dysfunction and loss of ECM synthesis and turnover. In fact, the loss of ECM-to-cell connection might start a vicious downwards spiral. For instance, during aging there is an increase in activity of ECM degrading enzymes, such as matrix metalloproteases (MMP) [7]. Increased MMP activity leads to collagen and ECM fragmentation, causing cell detachment, which leads to altered integrin signaling and an increase in mitochondrial reactive oxygen species, which in turn promotes the expression of more MMPs, leading to further ECM fragmentation [7]. Since cells synthesize the ECM (Figure 1A), this self-amplifying downwards spiral might underlie the observed decline in collagen production during aging across species [3,8,9]. For instance, collagen mass continuously declines at a rate of 1% per year in the human skin [10], as best illustrated by wrinkles and sagging skin. In general, during aging there is a progressive decline in ECM biosynthesis, accompanied by an increase in ECM degradation. There are two striking pieces of evidence suggesting that this loss of ECM biosynthesis and increase in ECM degradation might drive or accelerate aging. First, treating young human skin samples with MMPs quickly alters the skin morphology to look like old skin [7], and second deficits in an ECM-remodeling enzyme MMP14 in the skin leads to premature aging, short lifespan, and cellular senescence in mice [11]. MMPs cleave collagens. We might expect that mice genetically engineered to express modified collagen COL1A1$^{r/r}$ that cannot be cleaved by MMPs, would not show age-depended ECM degradation and would live longer. However, collagen COL1A1$^{r/r}$ mice showed accelerated aging, short lifespan, and cellular senescence [12], suggesting that blocking of ECM turnover is also detrimental. This points towards a balanced ECM turnover being important to maintain health during aging. Moreover, mouse models with premature aging disease (Hutchinson-Gilford Progeria Syndrome) fail to produce a functional ECM and their progeric cellular phenotypes and short lifespan are rescued by a functional ECM [13]. This suggest an integrated system of ECM-to-cell-to-nucleus linkage, translating outside mechanical forces to changes in cellular gene transcription to balance ECM and tissue homeostasis. Taken together, fragmentation or gradual weakening of the ECM, signals to cause alterations in mechanotransduction to the nucleus, thereby initiating a self-amplifying process, disrupting cell-ECM interaction and accelerating aging.

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Collagen glycation and crosslinking during aging

The Maillard reaction is the major non-enzymatic glycation pathway for advanced glycation end-products (AGE) formation [4]. Simplistically described, glucose interacts with lysine and arginine side chains of a protein (e.g., collagen) to form a reversible Schiff base product within hours, which is stabilized to keto amine (Amadori product) within days, and in a series of chemical reactions within weeks to months these are transformed into AGE (Figure 1B), such as carboxymethyl-lysine, 3-deoxyglucosone-lysine dimer, glyoxal, glucosepane, pentosidine etc [4]. These AGEs are able to crosslink and thus stiffen collagen structures. Glycation and crosslinking of collagens impairs wound healing and is a major problem for diabetes patients with high blood sugar levels [4]. Cells sense this increase in ECM stiffness and respond by expressing ECM-remodeling enzymes to adjust to their preferred matrix stiffness. However, AGEs or crosslinks hinder enzymatic cleavage, potentially leaving these stiffer matrix parts, but cleave the surrounding intact matrix resulting in collateral damage. Ultimately, this leads to stiffer and mechanically weaker matrices during aging.

Accumulation of protein aggregation in the ECM

Aggregation of proteins is a hallmark of many neurodegenerative diseases and is thought to be a crucial factor in their pathogenesis. Post-mortem studies of brains from Parkinson's patients show an intracellular aggregation of α-synuclein. Recent evidence suggests that α-synuclein aggregates might be actively secreted or released after cell death and taken up by other neurons to spread α-synuclein aggregation in a "prion-like fashion" throughout the brain. Injecting mice with antibodies against α-synuclein helps to clear extracellular α-synuclein [14]. Importantly, this kind of "prion-like" spreading has been observed in models for Tau pathologies or models for SOD1 mutant amyotrophic lateral sclerosis. Interestingly, targeting and clearing extracellular SOD1 by intraventricular infusion of a SOD1-specific antibody delayed the disease-onset and increased the lifespan of these SOD1(G93A) transgenic mice [15]. The accumulation of extracellular amyloid peptide (Aβ) has been suggested to be involved in the etiology of neurodegeneration in Alzheimer's disease [16]. How these extracellular aggregates are cleared and how these mechanisms directing this are lost during aging is still unclear. Microglia ("brain macrophages") and autophagy have

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been implicated in the clearance of extracellular aggregates. However, components of the extracellular matrix itself, such as collagen COL25A1, have emerged from whole-genome sequencing of healthy elderly people to be protective against Aβ pathology [17]. Thus, the ECM might not only be a by-stander that accumulates these aggregate-prone proteins, but might also have a functional role in either safe-storing or clearing these aggregates (Figure 1C). Alternatively, these aggregate-prone proteins in the ECM might turnover during the physiological ECM process (Figure 1C), which is progressively lost during aging.

Longevity-assurance pathways activate collagen turnover to protect against the progressive decline of ECM integrity during aging

Aged ECMs are characterized by a loss of collagen mass, accumulation of AGEs and protein aggregates, are stiffer, and fragmented, losing their protective and mechanical function important for cellular integrity. Many interventions that increase lifespan of *C. elegans*, prolong the expression and synthesis of collagens during aging [9]. Accompanying this prolonged-collagen-synthesis are MMPs and other enzymes, suggesting a proper integration and remodeling of collagens into the ECM [9]. This prolonged expression of collagens is required to extend lifespan of longevity-interventions, such as reduced Insulin/IGF-1 signaling, reduced TOR signaling, germ-stem cell ablation, and dietary restriction [9]. Furthermore, overexpression of certain key collagens is sufficient to increase lifespan of *C. elegans* [9]. Supplementing the diet with collagen peptides increases collagen expression and lifespan of *C. elegans* [18] and rats [19,20]. In human osteoblasts, supplementation with collagen peptides increases collagen expression [21]. It is unknown how collagen supplementation or collagen overexpression induces *de-novo* collagen biosynthesis and how this promotes longevity. I speculate that certain collagen levels or their integrity are monitored as indicators to maintain collagen and ECM homeostasis.

Rejuvenation capacity of young ECM

A fascinating finding is that placing senescent cells [22] or aged stem cells [23] in a "younger ECM" has been shown to rejuvenate these old cells. The ECM provides instructive signals that change cellular function and identity. For instance, placing tumor cells into an embryonic ECM, reprograms them to lose their tumorigenicity [24].

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Furthermore, the lost regenerative potential of old muscles is rejuvenated by grafting them into young, but not old hosts [25,26]. Heterochronic parabiosis, i.e., stitching together a young with an old circulatory system, or simply injecting young blood plasma has been shown to rejuvenate several organs of old mice. Moreover, administrating human umbilical cord plasma, which is enriched in TIMP2, rejuvenates the brain of old mice [27]. Tissue inhibitor of metalloproteinase TIMP2 inhibits MMPs, which suggest that alteration of the ECM might underlie this rejuvenation. Although the underlying rejuvenating mechanisms are unknown, these findings point towards the importance of maintaining ECM integrity for healthy aging. This raises the question of what constitutes a "young" ECM? Which are the key ECM components important to maintain cellular homeostasis and promote health during aging? In order to answer these questions, we first need to identify the key molecular components, modifications, and proteins that make up a "young or healthy ECM". Recent advances in the field of proteomics and data analysis enable such a quantification of ECM protein compositions. I build on these advances to define a novel concept that will help grasping changes in ECM composition during disease, health, aging, and longevity.

The Matrisome is the proteome of extracellular matrices

The entire set of proteins that can be expressed by the genome is known as the proteome. The proteome of extracellular matrices has been recently defined for humans, mice, and *C. elegans* and is called the "matrisome" (http://matrisomeproject.mit.edu; [28,29]). Thus, the matrisome is a subset of the entire proteome dedicated to extracellular matrices [28]. The matrisome is divided into two main categories: core- and associated- matrisome [28]. The core-matrisome is the set of proteins that are synthesized and secreted by cells to form extracellular matrices. The human genome encodes 44 collagen genes, 195 ECM glycoproteins (including, fibronectins, laminins etc), and 35 proteoglycans, forming the core-matrisome [28]. The "associated-matrisome" comprises of proteins that are secreted and either localize to the ECM or remodel the ECM. Thus, the "associated-matrisome" is further divided into secreted factors localizing to ECMs (344 proteins, including TGFβ, BMPs, Wnts, cytokines), ECM-regulators (238 proteins, including the proteases MMPs and cathepsins, the ECM-crosslinkers lysyl oxidases and transglutaminases), and ECM-affiliated (171proteins, including annexins, semaphorins, syndecans,

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glypicans, C-type lectins) [28]. The matrisome of humans comprises of 1027 genes, of mice 1110 genes, and of *C. elegans* 719 genes, which for each of these organisms is about 4% of their protein-encoding genome [28,29]. The mouse and human matrisome are quite similar, whereas the evolutionary more distant nematode *C. elegans* shares about 45% of conserved matrisome proteins with humans [29]. These include proteins usually found in metazoans, such as laminins, collagens type IV, XVIII, and XXV, perlecans, and syndecans [29]. For instance, human COL25 associated with healthy aging [17] is *col-99* in *C. elegans* [29]. This *in-silico* definition and comprehensive compendium of ECM proteins is key for the analysis of transcriptomic and proteomic datasets in order to identify matrisome gene products associated with young ECM and/or with healthy aging.

The matreotype is the state of the matrisome associated with a phenotype

The matrisome encompasses all the ECM and ECM-associated proteins that are or can be expressed by a genome. However, different cell types express different ECM proteins [3]. Organismal phenotypes and physiological stages are characterized by distinct sets of expressed ECM proteins and by the occurrence of different posttranslational protein modifications [3]. Thus, the compositions and modifications of different matrisome proteins reflect cellular identity, physiological status, and phenotype. Based on this, I propose here a new term: the “matreotype”, which is the acute state of an ECM composition and/or modification associated with or causal for a given physiological condition or phenotype. Similar to the proteotype reflecting a specific state of the proteome [30], the matreotype reflects a specific state of the matrisome linked to a phenotype.

Below, I discuss technical challenges and theoretical considerations of the matreotype. As a proof-of-concept, we re-analyzed gene expression and proteomics data to identify a preliminary matreotype during aging and longevity, demonstrating that by combining transcriptomic, proteomic, and genetic approaches, the matreotype of healthy aging can be revealed across species in future approaches. Furthermore, I highlight that identifying and defining matreotypes will be of translational value.

Theoretical considerations of matreotypes during aging and longevity

Aging is characterized by a progressive physiological decline over time. This physiological decline is reflected in the matreotype as for instance in changes in

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composition of ECM proteins, accumulation of AGEs on collagens, and collagen crosslinking. During development and growth, cells constantly remodel the ECM by degrading parts of the ECM and through *de-novo* synthesis of matrisome components in order to maintain homeostasis. This dynamic and energy-intensive process might decline after reproduction. Either because natural selection, as defined as reproductive fitness, is ineffective after reproduction, or because of a shift in resource allocation from somatic to germline tissue during the onset of reproduction. Irrespective of the etiology, this predicts that after reproduction the homeostasis of matrisome components, i.e. *de-novo* synthesis and ECM remodeling, would decline and should be reflected in the temporal change of matreotypes during aging. Thus, the matreotype of a young ECM is different compared to an old ECM. The gradual decline of ECM over time should be reflected by distinct matreotypes at any given time point during aging. This raises the question whether in long-lived animals this gradual decline in matreotype is simply slowed during aging (temporal scaling) or longevity-assurance pathways produce an alternate matreotype to maintain youthfulness? The alternate matreotype could be conceptually like the Waddington model during development; i.e., just an alternative or parallel path or fate during aging. Either model, temporal scaling or alternate path/fate, would have implications on how to design strategies to improve health during aging. If its temporal scaling, interventions might be started right after reproduction to slow down this progressive decline in physiology. If it is an alternate fate, it could be activated during old age, assuming that there has not been excessive damage accumulation in order to promote some cellular function and reprogramming during aging.

Technical challenges for quantifying matreotypes

There are several technical challenges for quantifying matreotypes that in principle could be overcome. First, quantifying the matreotype through standard proteomics needs to be adopted to enrich for ECM proteins. Since ECM proteins are crosslinked and form high molecular networks, they become insoluble and this insoluble fraction is often discarded. However, there are currently several proteomic protocols that have been successfully used to enrich for ECM proteins [31]. Furthermore, a targeted proteomics approach is needed for the quantification and reproducibility, such as SWATH-MS (https://imsb.ethz.ch/research/aebersold/research/swath-ms.html) or other comparable approaches. Second, although mRNA levels might be indicative for

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de-novo synthesis of ECM proteins, mRNA expression levels largely do not reflect protein levels, which is very likely to be true for ECM proteins. For instance, even if a collagen is synthesized, correctly modified, forms a triple helix, and is secreted, this collagen needs to be further processed and incorporated into the matrix. Otherwise these soluble collagens accumulate amorphously in the extracellular space as seen in fibrosis. Since collagens can act as a glue ("*kolla*" means glue, "*gen*" means producing), this property of filling the extracellular space is important for wound closure. Moreover, inflammation proceeds fibrosis [32] and during aging there is a steady increase of inflammation. This inflammation-induced matreotype, which is characteristic for fibrosis and amorphous collagen deposition during aging can be assessed through tissue staining and biochemical techniques [33]. A third aspect for consideration is that there is also heterogeneity within cell types and tissues due to randomness in cellular gene expression including the expression and biosynthesis of ECM proteins. While single cell RNA-sequencing is feasible, single cell proteomics is more challenging. Alternatively, tagging ECM proteins with fluorescent proteins could reveal the matreotypes during aging *in vivo* and *non-invasively* [34]. Thus, these few examples already indicate that it might be challenging but feasible to quantify and interpret matreotypic changes during aging. A possible strategy would be to focus on functional or "causal" matreotypic changes during aging, which need to be experimentally identified and validated.

Matreotype during aging

A systematic and longitudinal quantification of matreotype during aging or longevity is missing. However, here I present a preliminary matreotype by re-analysing publicly available expression profiles and a proteomics dataset. Budovskaya and colleagues [35] measured the mRNA expression levels during *C. elegans* aging (day 0-11 of adulthood). They found that of the approximately twenty thousand *C. elegans* genes, about 900 genes decline and 300 are increased in expression during aging [35]. To re-analyze this dataset, we used the *C. elegans* Matrisome Annotator (http://ce-matrisome-annotator.permalink.cc/; [29]). About 150 out of these 1254 age-regulated genes [35] are matrisome genes, comprising of 92 collagen genes (Figure 2A, 2B). Most matrisome genes are expressed during development and growth, but their expression rapidly declines during the reproductive phase (day 1-4 of adulthood; Figure 2C). There are only three matrisome-associated genes that increase in

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expression during aging (*cpr-2*, *chil-14, lec-2*; Figure 2C). CPR-2 is the cathepsin B (CTSB) orthologue and might be involved in ECM degradation. Consistent with observations in mammals, tissue inhibitor of metalloproteases, TIMP-1, expression is also progressively lost during aging (Figure 2C). Taken together, we found a general decline of matrisome gene expression during aging for the model organism *C. elegans* (Figure 2C) that is similar to the progressive decline in collagen biosynthesis observed in human skin [10] and across species [8,9]. A comprehensive assessment of not only the transcriptional matreotype but also of the protein levels in mammalian tissues during aging might be feasible by using an *in-situ* decellularization protocol coupled with ECM proteomics [36]. This could reveal the longitudinal signature of the matreotype during aging.

Matreotype during longevity

Comparing the gene expression or protein levels at a given chronological age between wild type and long-lived animals revealed several molecular mechanisms at work, which were then experimentally assessed for their functional importance for longevity. For instance, reduced Insulin/IGF-1 signaling, upregulates genes involved in antimicrobial, oxidative stress and xenobiotic responses, protein homeostasis, and metabolism, all of which are required to promote healthy aging [37]. Re-analyzing the expression profile of these long-lived mutants with reduced Insulin/IGF-1 signaling [9], revealed that almost one-fifth, i.e., 79 of the 426 upregulate genes are matrisome genes (See Supplementary Table 5 of [29]). Out of the 79 upregulated matrisome genes, 48 are collagens and 15 are ECM proteases (cathepsins, astacin-like metalloendopeptidases, MMPs) [29], suggesting an activation of collagen remodeling [9]. Such a mobilization of matrisome genes also occurs through other longevity-assurance pathways. Re-analyzing the proteomics dataset comparing long-lived germ stem cell mutants (*glp-1*) with wild-type *C. elegans* [38], revealed an increase of 177 proteins including 25 matrisome proteins in long-lived *C. elegans* (Supplementary Table 6 of [29]). The 25 matrisome proteins include two basement membrane-forming laminins, ten collagens, one prolyl 4-hydroxylase (DPY-18), which is important for collagen stability, and three ECM-remodeling enzymes (Supplementary Table 6 of [29]), suggesting an increase in ECM turnover and homeostasis. Surprisingly, I could not find any proteomics attempts comparing long-lived mice with wild type during aging. Whether longevity-assurance pathways in mammals alter the matreotype

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towards re-activating ECM homeostasis needs to be investigated. Taken together, based on the data from *C. elegans*, it appears that longevity-assurance pathways invest in collagen or ECM turnover to maintain a youthful matreotype.

Clinical implications for the matreotype

The physiological state of a cell or tissue is reflected in an unique ECM composition or matreotype [2]. For instance, fibroblasts that become senescent or are dedifferentiated into myofibroblasts or cancer-associated fibroblast express a distinct set of ECM proteins. Based on the ECM signature or matreotype, it is even possible to identify tumor type [39]. Thus, distinct matreotypes could be developed into biomarkers or prognostic indicators for disease and health status with implications for personalized medicine. Targeting ECM-cell surface receptors might provide an entry point to remodel ECM and matreotype, since cell-surface receptors read-out ECM stiffness and ECM properties to reprogram cells. Biologics or antibodies that act only on the cellular surfaces could target and alter these ECM-cell surface receptors to change intracellular signaling and to induce the desired gene expression program. Currently, there are about 11 different targets being investigated in 27 clinical trials with primary end points specific to ECM stiffness [40] that might reprogram the matreotype.

Conclusions

Major efforts have revealed how proteins are maintained within cells and cellular compartments and how longevity interventions improve protein homeostasis during aging (Figure 1). Here, I propose that collagen homeostasis or ECM turnover is a process that works efficiently when the organisms are young to maintain their somatic tissue. Since collagen biosynthesis is costly and energy-intensive, upon reproduction resources might be allocated to produce high quality off-springs. Moreover, ECM turnover might be the repair mechanism to cleave-out, digest, and degrade damaged ECM proteins from the matrix. This requires cellular contact, proper mechanotransduction, and *de-novo* synthesis of ECM components from cells. Damage to the ECM or to cells will start a vicious downwards spiral of ECM fragmentation and cell-detachment leading to a progressive decline in cellular and ECM homeostasis during aging. Longevity interventions might also maintain protein homeostasis of extracellular proteins reflected in changes of the matreotype (Figure

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1). Thus far, we have taken the first steps to define the matrisome and the matreotypes during aging and longevity (Figure 2). Since the matreotype reflects the cellular, tissue, and disease status, quantifying and defining the matreotype could become a valuable biomarker for health assessment. Rejuvenating the matreotype might systemically rejuvenate cellular and tissue functions. Identifying druggable targets and understanding how to trigger rejuvenation of the aged matreotype has broad implications for clinical applications.

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Figures

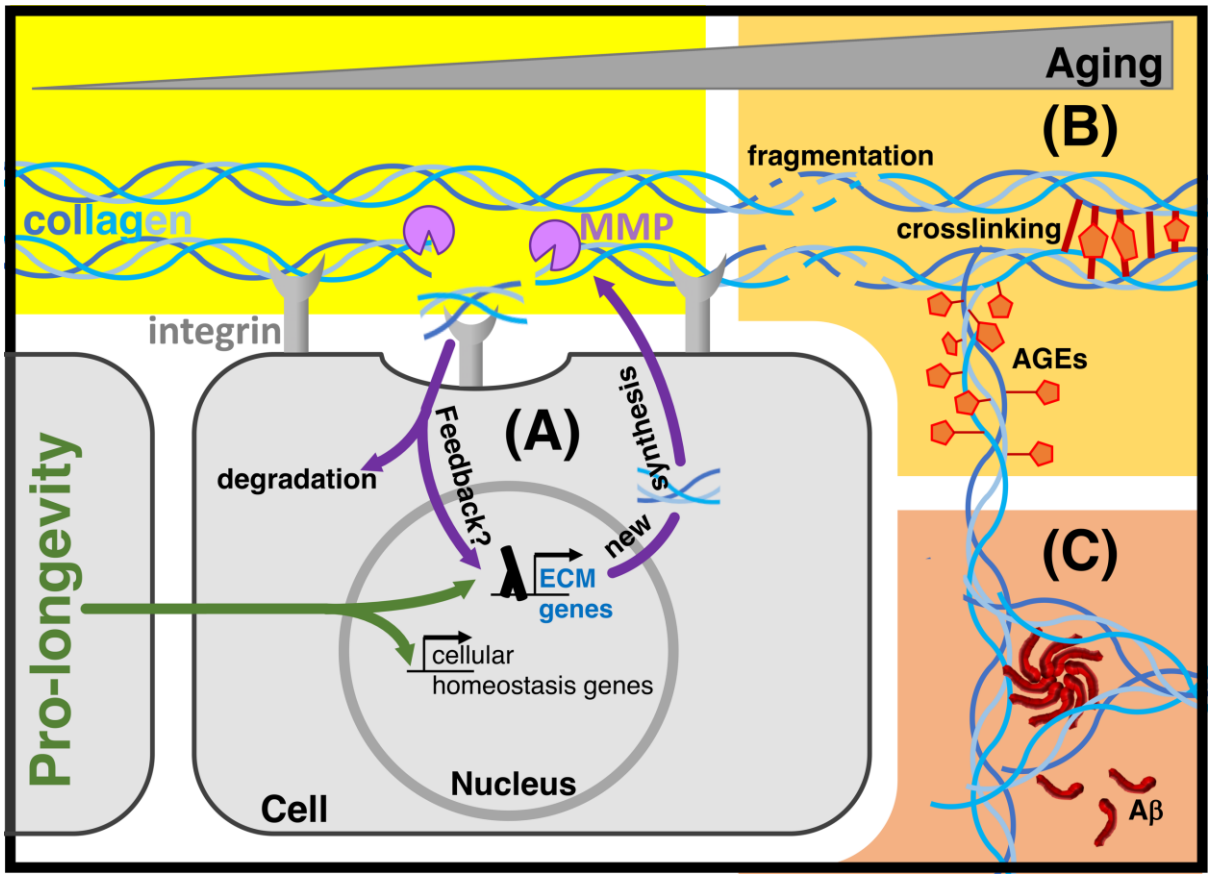

Figure 1. Hypothetical model of age-related damages to extracellular matrices.

A) Cells synthesize and secrete proteins that form extracellular matrices (ECM). Cells are anchored to the ECM via cell-surface receptors, such as integrins. Integrins and other receptors transduce mechanical information via biochemical intracellular signaling cascades or cytoskeleton linkages to the nucleus. Matrix metalloproteases (MMP) cleave collagens and other proteins from the ECM. Pro-longevity interventions not only improve cellular protein homeostasis, but might also improve extracellular protein homeostasis.

B) During aging, the ECM becomes fragmented, glycated, modified by advanced glycation end products (AGE), and/or crosslinked.

C) Aggregation prone peptides, such as amyloid beta (Aβ), accumulate in extracellular matrices.

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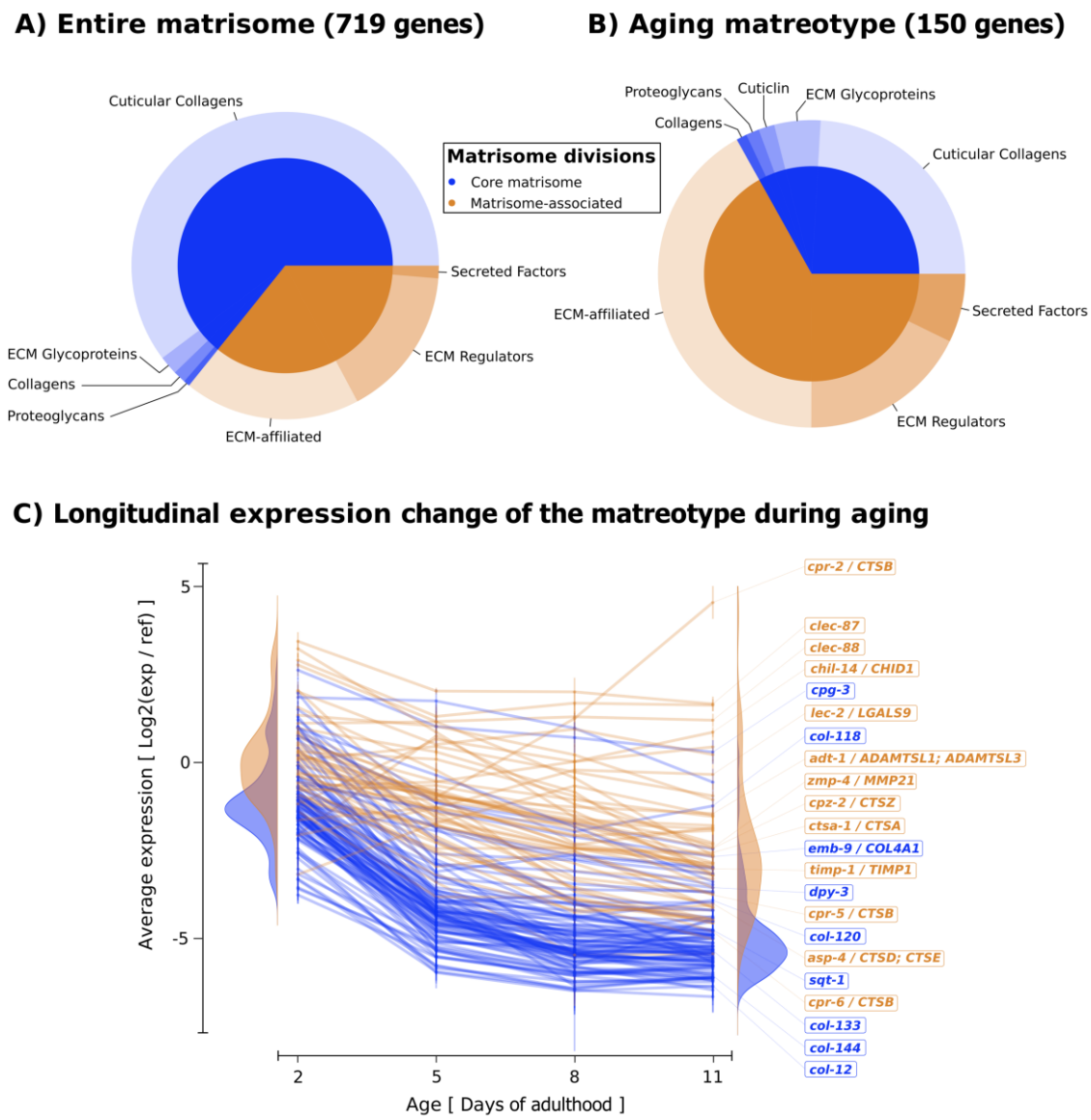

Figure 2. Changes in the matreotype during aging.

A) Schematic representation of the entire matrisome of *C. elegans*.

B) The aging matreotype consists of 150 differentially expressed matrisome genes during *C. elegans* aging.

C) Longitudinal expression profile of the 150 matrisome genes undergoing significant age-dependent expression changes.

Core matrisome and corresponding subcategories in shades of blue and matrisome-associated categories in shades of organ. Expression dataset from Budovskaya *et al., Cell* 2008 re-analyzed with the *C. elegans* Matrisome Annotator (http://ce-matrisome-annotator.permalink.cc/ developed by Cyril Statzer (Teuscher *et al., Matrix Biology Plus* 2019).

 doi:10.20944/preprints201908.0063.v1

References

1. Hynes RO: The extracellular matrix: not just pretty fibrils. Science 2009 Nov 27;326:1216–1219.

2. Bonnans C, Chou J, Werb Z: Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 2014 Dec;15:786–801.

3. Frantz C, Stewart KM, Weaver VM: The extracellular matrix at a glance. Journal of Cell Science 2010 Dec 15;123:4195–4200.

4. Birch HL: Extracellular Matrix and Ageing; in : Biochemistry and Cell Biology of Ageing: Part I Biomedical Science. Singapore, Springer Singapore, 2019, pp 169–190.

5. Toyama BH, Hetzer MW: Protein homeostasis: live long, won't prosper. Nat Rev Mol Cell Biol 2013 Jan;14:55–61.

6. Kjaer M, Langberg H, Miller BF, Boushel R, Crameri R, Koskinen S, et al.: Metabolic activity and collagen turnover in human tendon in response to physical activity. J Musculoskelet Neuronal Interact 2005 Mar;5:41–52.

7. Fisher GJ, Quan T, Purohit T, Shao Y, Cho MK, He T, et al.: Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. American Journal Of Pathology 2009 Jan;174:101–114.

8. de Magalhães JP, Curado J, Church GM: Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics 2009 Apr 1;25:875–881.

9. Ewald CY, Landis JN, Porter Abate J, Murphy CT, Blackwell TK: Dauer-independent insulin/IGF-1-signalling implicates collagen remodelling in longevity. Nature 2015 Mar 5;519:97–101.

10. Shuster S, Black MM, McVitie E: The influence of age and sex on skin thickness, skin collagen and density. Br J Dermatol 1975 Dec;93:639–643.

11. Gutiérrez-Fernández A, Soria-Valles C, Osorio FG, Gutiérrez-Abril J, Garabaya C, Aguirre A, et al.: Loss of MT1-MMP causes cell senescence and nuclear defects which can be reversed by retinoic acid. EMBO J 2015 Jul 14;34:1875–1888.

12. Vafaie F, Yin H, O'Neil C, Nong Z, Watson A, Arpino J-M, et al.: Collagenase-resistant collagen promotes mouse aging and vascular cell senescence. Aging Cell 2013 Sep 19;13:121–130.

13. Hernandez L, Roux KJ, Wong ESM, Mounkes LC, Mutalif R, Navasankari R, et al.: Functional coupling between the extracellular matrix and nuclear lamina by Wnt signaling in progeria. Dev Cell 2010 Sep 14;19:413–425.

14. Bae EJ, Lee HJ, Rockenstein E, Ho DH, Park EB, Yang NY, et al.: Antibody-Aided Clearance of Extracellular -Synuclein Prevents Cell-to-Cell Aggregate Transmission. J Neurosci 2012 Sep 26;32:13454–13469.

15. Urushitani M, Ezzi SA, Julien J-P: Therapeutic effects of immunization with mutant superoxide dismutase in mice models of amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 2007 Feb 13;104:2495–2500.

16. Selkoe DJ: The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. Trends Cell Biol 1998 Nov;8:447–453.

17. Erikson GA, Bodian DL, Rueda M, Molparia B, Scott ER, Scott-Van Zeeland AA, et al.: Whole-Genome Sequencing of a Healthy Aging Cohort. Cell 2016 May 5;165:1002–1011.

18. Morikiri Y, Matsuta E, Inoue H: The collagen-derived compound collagen tripeptide induces collagen expression and extends lifespan via a conserved p38 mitogen-activated protein kinase cascade. Biochemical and Biophysical Research Communications 2018 Nov 10;505:1168–1173.

19. Liang J, Pei X-R, Wang N, Zhang Z-F, Wang J-B, Li Y: Marine collagen peptides prepared from chum salmon (Oncorhynchus keta) skin extend the life span and inhibit spontaneous tumor incidence in Sprague-Dawley Rats. J Med Food 2010 Aug;13:757–770.

20. Liang J, Pei X, Zhang Z, Wang N, Wang J, Li Y: The protective effects of long-term oral administration of marine collagen hydrolysate from chum salmon on collagen matrix homeostasis in the chronological aged skin of Sprague-Dawley male rats. J Food Sci 2010 Oct;75:H230–8.

21. Tsuruoka N, Yamato R, Sakai Y, Yoshitake Y, Yonekura H: Promotion by collagen tripeptide of type I collagen gene expression in human osteoblastic cells and fracture healing of rat femur. Biosci Biotechnol Biochem 2007 Nov;71:2680–2687.

22. Choi HR, Cho KA, Kang HT, Lee JB, Kaeberlein M, Suh Y, et al.: Restoration of senescent human diploid fibroblasts by modulation of the extracellular matrix. Aging Cell 2011 Feb;10:148–157.

23. Sun Y, Li W, Lu Z, Chen R, Ling J, Ran Q, et al.: Rescuing replication and osteogenesis of aged mesenchymal stem cells by exposure to a young extracellular matrix. The FASEB Journal 2011 May;25:1474–1485.

24. Hendrix MJC, Seftor EA, Seftor REB, Kasemeier-Kulesa J, Kulesa PM, Postovit L-M: Reprogramming metastatic tumour cells with embryonic microenvironments. Nat Rev Cancer 2007 Apr;7:246–255.

25. Carlson BM, Faulkner JA: Muscle transplantation between young and old rats: age of host determines recovery. Am J Physiol 1989 Jun;256:C1262–6.

26. Carlson BM, Dedkov EI, Borisov AB, Faulkner JA: Skeletal muscle regeneration in very old rats. J Gerontol A Biol Sci Med Sci 2001 May;56:B224–33.

27. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, et al.: Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature 2017 Apr 27;544:488–492.

28. Naba A, Clauser KR, Ding H, Whittaker CA, Carr SA, Hynes RO: The extracellular matrix: Tools and insights for the “omics” era. Matrix Biol 2016 Jan;49:10–24.

29. Teuscher AC, Jongsma E, Davis MN, Statzer C, Gebauer JM, Naba A, et al.: The *in-silico* characterization of the *Caenorhabditis elegans* matrisome and proposal of a novel collagen classification. Matrix Biology Plus 2019 Mar 11;:1–13.

30. Aebersold R, Mann M: Mass-spectrometric exploration of proteome structure and function. Nature 2016 Sep 15;537:347–355.

31. Naba A, Clauser KR, Hynes RO: Enrichment of Extracellular Matrix Proteins from Tissues and Digestion into Peptides for Mass Spectrometry Analysis. J Vis Exp 2015;:e53057.

32. Wick G, Grundtman C, Mayerl C, Wimpissinger T-F, Feichtinger J, Zelger B, et al.: The Immunology of Fibrosis. Annu Rev Immunol 2013;31:107–135.

33. Teuscher AC, Statzer C, Pantasis S, Bordoli MR, Ewald CY: Assessing Collagen Deposition During Aging in Mammalian Tissue and in *Caenorhabditis elegans*. Methods Mol Biol 2019;1944:169–188.

34. Teuscher AC, Ewald CY: Overcoming Autofluorescence to Assess GFP Expression During Normal Physiology and Aging in *Caenorhabditis elegans*. BIO-PROTOCOL 2018 Jul 20;8. DOI: 10.21769/BioProtoc.2940

35. Budovskaya YV, Wu K, Southworth LK, Jiang M, Tedesco P, Johnson TE, et al.: An elt-3/elt-5/elt-6 GATA Transcription Circuit Guides Aging in *C. elegans*. Cell 2008 Jul 25;134:291–303.

36. Mayorca-Guiliani AE, Madsen CD, Cox TR, Horton ER, Venning FA, Erler JT: ISDoT: in situ decellularization of tissues for high-resolution imaging and proteomic analysis of native extracellular matrix. Nat Med 2017 Jun 12;23:890–898.

37. Ewald CY, Castillo-Quan JI, Blackwell TK: Untangling Longevity, Dauer, and Healthspan in *Caenorhabditis elegans* Insulin/IGF-1-Signalling. Gerontology 2018;64:96–104.

38. Pu Y-Z, Wan Q-L, Ding A-J, Luo H-R, Wu G-S: Quantitative proteomics analysis of *Caenorhabditis elegans* upon germ cell loss. J Proteomics 2017 Mar 6;156:85–93.

 doi:10.20944/preprints201908.0063.v1

39. Socovich AM, Naba A: The cancer matrisome: From comprehensive characterization to biomarker discovery. Seminars in Cell & Developmental Biology 2019 May;89:157–166.

40. Lampi MC, Reinhart-King CA: Targeting extracellular matrix stiffness to attenuate disease: From molecular mechanisms to clinical trials. Sci Transl Med 2018 Jan 3;10:eaao0475.