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Lamin-B in systemic inflammation, tissue homeostasis, and aging

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Gradual loss of tissue function (or homeostasis) is a natural process of aging and is believed to cause many age-associated diseases. In human epidemiology studies, the low-grade and chronic systemic inflammation in elderly has been correlated with the development of aging related pathologies. Although it is suspected that tissue decline is related to systemic inflammation, the cause and consequence of these aging phenomena are poorly understood. By studying the *Drosophila* fat body and gut, we have uncovered a mechanism by which lamin-B loss in the fat body upon aging induces age-associated systemic inflammation. This chronic inflammation results in the repression of gut local immune response, which in turn leads to the over-proliferation and mis-differentiation of the intestinal stem cells, thereby resulting in gut hyperplasia. Here we discuss the implications and remaining questions in light of our published findings and new observations.

Keywords: aging, lamin-B, fat body, gut hyperplasia, heterochromatin, inflammation, lamin-associated chromatin domains (LADs), tissue homeostasis

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Introduction

Much progress has been made in understanding how alterations in signaling pathways, transcriptional networks, and epigenetic modifications can contribute toward the process of aging in different organisms. One of the challenges is to understand how these alterations lead to the dysfunction of cell structures and how the defective cell structure leads to the aging phenotypes. The chronic low level of systemic inflammation in the absence of any apparent infection in elderly humans represents a well-established aging phenotype.[1,2] This age-associated chronic inflammation, often referred to as inflammaging, is defined as the elevated circulating pro-inflammatory cytokines, including interleukin (IL)-6, IL-1, and tumor necrosis factor α (TNFα).[3-5] Some epidemiology studies suggest that individuals exhibiting the inflammaging phenotype have an increased probability of developing several aging associated pathologies and subsequent death.[6,7] Consistent with this, the extremely long-lived individuals, the centenarians, have a significantly reduced inflammaging phenotype as compared to the general aging population.[8]

To establish how the known aging pathways contribute to inflammaging and age-associated pathologies, it is essential to first understand why the aging human body secretes more circulating pro-inflammatory cytokines than that of the young and whether these cytokines directly contribute to disease. One of the often-discussed ideas is that the age-associated tissue degeneration would induce inflammation and mis-regulated repair. However, it is also possible that some cell-intrinsic structural changes upon aging could result in the de-repression of inflammatory proteins or genes. Unfortunately, since very little is known about what kind of age-associated changes occur inside cells, it is difficult to pinpoint the cause of systemic inflammation. In addition to the difficulty to decipher why aging leads to systemic inflammation, the consequence of inflammaging is also challenging to establish because epidemiology studies can only indicate a correlation between inflammaging and the increased incidence of diseases. Establishing the causal relationship between inflammaging and disease would require additional mechanistic studies.

Using *Drosophila melanogaster*, a much simpler organism than mammals, we have uncovered how one nuclear defect, the

loss of lamin-B due to aging, leads to systemic inflammation and disruption of tissue homeostasis.[9] We will discuss our findings in the context of tissue building and maintenance. We will also discuss the importance of linking the known aging pathways with the regulation of the integrity of the nucleus.

Nuclear lamins in tissue building and maintenance

Nuclear lamins are the type V intermediate filament proteins encoded by one or up to 3 genes depending on the organism. For example, mammals have 3 lamin genes encoding one A-type lamin called lamin-A and 2 B-type lamins called lamin-B1 and -B2, whereas *Drosophila* has 2 lamin genes encoding the A- and B-type lamins called LAMC and LAM, respectively. Based largely on the study using tissue culture cells, lamins are known to assemble into a dense meshwork underneath the inner nuclear envelope where they interact with the chromatin and many nuclear peripheral proteins, including the nuclear pore complex (NPC) and the inner nuclear envelope proteins. Considering these multitudes of interactions, it is not surprising that lamins have been found to have a large number of nuclear functions, such as chromatin organization, transcriptional regulation, and nuclear shape maintenance.[10-12] Consequently, lamins have been viewed as housekeeping proteins essential for basic cell viability.

More recent studies, however, have shown that mouse embryonic stem cells (mESCs) deleted of all 3 lamin genes can self-renew and undergo differentiation *in vitro*.[13] This demonstrates that lamins are not required for the survival of at least one cell type, the mESCs. By analyzing the role of lamins in model organisms, it is now clear that these proteins are required for the proper development of multiple organs, including the brain, the diaphragm, and the testis.[14-17] The mechanism by which lamins function in organ building is not well understood, but the knowledge on how lamins function in tissue culture cells should facilitate the effort of deciphering the developmental role of these proteins. Indeed the finding that the *Drosophila* lamin-B is required for the testis development by regulating the EGF signaling in the cyst stem cells is aided by the knowledge that lamin-B interacts with some nucleoporins.[15]

In addition to the developmental functions, the studies of human diseases caused by mutations in lamins have suggested that these proteins are required for the proper function of organs and tissues. For example, point mutations in lamin-A have been shown to cause a number of human diseases, including cardiomyopathy, muscular dystrophy, partial lypodystrophy, and a premature aging disease called progeria.[18-20] In fact cells derived from progeria patients have been used as a model to study the cellular basis of premature aging. Together these studies beg the questions such as whether the loss of lamins occurs during natural aging and whether such loss leads to cell or tissue dysfunction.

Lamin-B loss in cells cultured *in vitro* and in old tissues

Studies using primary fibroblasts derived from humans and mice have shown that these cells have a finite replicative lifespan when cultured *in vitro*. Prolonged culturing leads to cellular senescence characterized by the cessation of cell division and changes in a large number of cell features, including increased secretion of inflammatory factors and the alteration of cell shape and chromatin organization. Interestingly, recent studies show that the senescence of mammalian fibroblasts *in vitro* is accompanied by the loss of lamin-B1.[21-23] The decline of lamin-B1 in these cultured cells appears to be regulated at both transcriptional and posttranscriptional levels. In fact, activation of either the Rb or the p53 tumor suppressor in the primary human fibroblasts is sufficient to lead to lamin-B1 reduction.[22] Lamin-B1 loss is also detected in fibroblasts derived from progeria patients and primary fibroblasts with shortened telomere or with other DNA damages.[22-24] Since irradiation-induced cell senescence in mice also leads to lamin-B1 loss, it is proposed that the decline of lamin-B1 *in vivo* could be used as a senescence biomarker.[22]

It is relatively difficult to quantify lamin protein levels in mammalian tissues and organs. So far, only human skin keratinocytes are reported to exhibit reduced lamin-B1 levels upon aging.[23] It remains unclear whether this loss is caused by the senescence of the old keratinocytes. By analyzing the lamin levels in different organs in young and old *Drosophila*, we found a clear age-associated loss of LAM in both fat bodies and malpighian tubules, but LAMC levels remained unchanged in these tissues (**Fig. 1**). Importantly, not all old tissues lose lamin upon aging. For example, the cells in the young and old gut exhibit similar levels of LAM and LAMC (**Fig. 1**). The gut epithelia undergo continuous replacement by the intestinal stem cells. The similar lamin levels in the young and old gut might be because the senescence cells are continuously replaced. However, the post mitotic

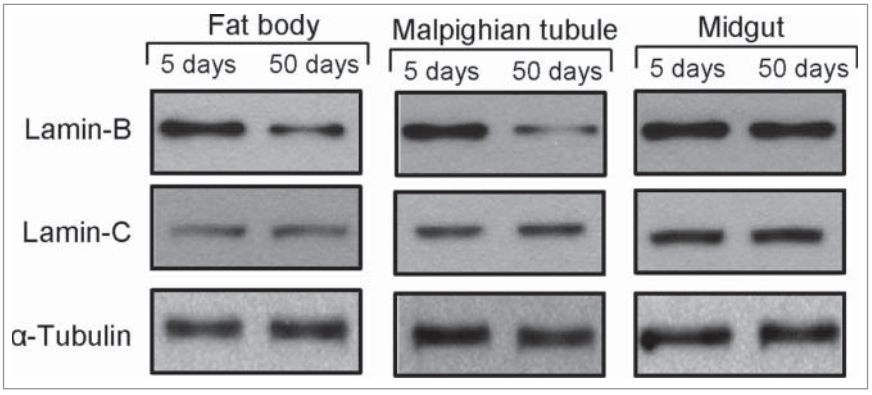

Figure 1. Reduction of lamin-B in the fat body and Malpighian tubule but not in the midgut upon aging. LAM loss in aged fat body and Malpighian tubules, but not in aged midgut, as revealed by Western blotting analyses of dissected fat bodies, Malpighian tubules, and midguts from 5- or 50-day old flies. LAMC level remained unchanged. α-Tubulin, loading control.

heart cells and oenocytes that are known to persist throughout the lifespan of flies also do not lose their lamins upon aging.[9] Therefore, it is important to further explore how lamin protein levels are regulated in different tissues and how this helps to maintain tissue homeostasis.

Lamin-B may maintain tissue function by controlling gene expression

To understand the role of lamin-B in tissue maintenance, we performed RNA-seq to compare the gene expression differences in young (5 days) and old (50 days) fat body and also in 5-day fat body in which LAM was depleted by fat body specific RNAi. Gene ontology (GO) analyses revealed that the age-associated LAM loss resulted in gene expression changes in a set of genes belonging to pathways essential for maintaining tissue functions (**Fig. 2A**).[9] Importantly, depletion of LAM in young fat body also caused the change of expression of a similar set of genes as observed in those old fat bodies (**Fig. 2B**). Further analyses revealed that LAM depletion resulted in either up or down regulation of genes belonging to the metabolic, proteolysis, and oxidative pathways. Interestingly, the immune response genes seem to be significantly up regulated, whereas genes required for cell fates determination and cell-cell adhesion appear to be significantly down regulated upon LAM loss in both old and young fat bodies. These findings suggest that lamin-B could either activate or repress genes important for fat body tissue integrity and function.

Based on DNA adenine methyltransferase identification (DamID) and chromatin immunoprecipitation (ChIP)-sequencing, the chromatin regions that associate with lamins have been mapped in a number of cell types.[14,25,26] By analyzing genes localized in the lamin-B1 associated chromatin domains (also called LADs) in different cell types, we found that the immune response genes were significantly enriched in the LADs. Since lamins are known to repress gene expression, it is possible that LAM in the *Drosophila* fat body could repress immune response in the absence of infection. Consistent with this idea, we found a clear global reduction of heterochromatin protein 1 (HP1) and histone H3 lysine 9 trimethylation (H3K9me3) in the wild type old fat bodies or the young fat bodies depleted of LAM. Moreover, an extra copy of HP1 partially reduced the inflammatory response caused by LAM reduction in the fat body. Importantly, ChIP-qPCR analyses of H3K9me3 in the fat body revealed that depletion of LAM reduced H3K9me3 on several immune response genes. Together our studies show that lamin-B represses the expression of the immune response genes in fat bodies by maintaining the heterochromatin.[9] Currently, it is not known how LAM may regulate the expression of genes required for metabolism, oxidation, cell fates, and cell-cell adhesion.

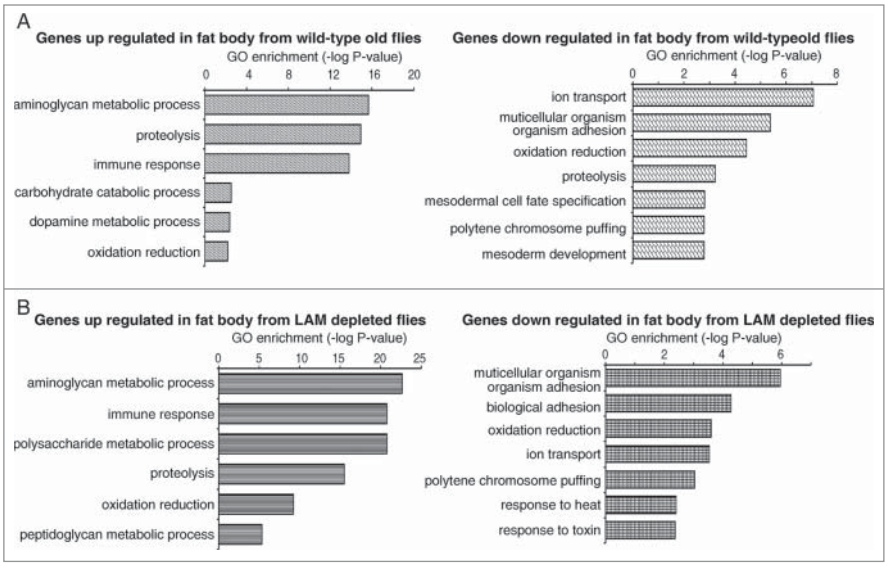

Figure 2. Gene Ontology (GO) analyses of old wild type fat body and young fat body depleted of lamin-B. GO were determined by DAVID Bioinformatics Resources 6.7. Shown are significant GO terms determined from genes that were up- or down-regulated by equal or greater than 2 fold in old fat bodies (**A**), 50-day, wild type w^{1118}) or young fat bodies depleted of lamin-B (**B**), 5-day, *Cg-Gal4/+;UAS-lamin-B RNAi/tub-Gal80*^{ts}).

Lamin-B loss in one tissue may disrupt the function of other tissues via systemic inflammation

By focusing on the study of one of the 2 innate immune response pathways, called the immune deficiency (IMD) pathway, we show that both the age-associated LAM loss in the old fat body and RNAi-mediated depletion of LAM in young fat body lead to a significant fat body inflammation mediated by the IMD signaling. This fat body inflammation represses the IMD activity in the midgut, which leads to gut hyperplasia. By analyzing the circulating peptidoglycan recognition proteins (PGRPs) that became derepressed upon LAM loss in the fat body, we found that these PGRPs contribute toward gut hyperplasia. Thus lamin loss in one tissue can affect the function of other tissues. In the case of fat body LAM loss, the disruption of the midgut is at least in part mediated by systemic inflammation.[9] Further studies will be needed to investigate how the fat body secreted PGRPs influence the gut immune system.

Nuclear deterioration and its connection to the known aging pathways

In addition to the decline of the nuclear lamin-B level as a result of natural aging in *Drosophila* and humans, previous studies have shown an age-associated disruption of the nuclear pore complex (NPC) in the post mitotic cells in *C. elegans*.[27] The age-associated NPC deterioration has been linked to defects such as nuclear leakage. The lack of exchange of several scaffold nucleoporins in the post mitotic cells is thought to contribute to the decay of the NPC upon aging, because without the exchange, these so-called long-lived proteins would accumulate

damages,[28] thereby leading to the structural defects of the NPCs. The damaged nucleoporins could be degraded in the old cells, but it remains unclear whether the protein levels of any nucleoporins were reduced upon aging. Interestingly, however, lamin appears to be slowly exchanged in the post mitotic nuclear lamina in old *C. elegans*.[27] Further studies that monitor protein exchange and protein levels in post mitotic cells at different aging stages in post mitotic cells should help to clarify whether aging is accompanied by the declined ability to repair the damaged proteins in large structures such as the nuclear lamina and the NPC.

Maintaining the homeostasis of cellular proteins, a process often referred to as proteostasis, is essential to the extension of lifespan induced by the known pathways including the dietary restriction, enhanced autophagy, and reduced insulin signaling.[29] Studies have shown the decline in cell's ability to manage the mis-folded and/or aggregated proteins occurs upon aging. It is possible that the nuclear proteins such as lamin-B and some NPCs are particularly sensitive to this decline. Since both lamin-B and the components of NPCs can regulate gene expression[30] and since lamin-B disruption can deregulate genes involved in cell/tissue homeostasis, even subtle damage of these proteins may lead to positive feedbacks that further promote the aging process. Considering that *Drosophila* is an excellent aging model, the lamin-B loss in the old fat body and its consequences we have uncovered should provide a great entry point to further dissect the crosstalk between the known pathways that affect aging and the maintenance of the nuclear protein homeostasis. Understanding this crosstalk should shed light on how the decline of the cell nucleus contributes toward age-associated diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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