

Extra View

Reversal of aging by NFκB blockade

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Abbreviations: IGF-1, insulin-like growth factor 1; ΔSP-p50-ER, dominant negative p50 fused to mutant estrogen receptor; 4-OHT, 4-hydroxytamoxifen

Key words: NFκB, longevity, senescence, skin, microarray, computational

Genetic studies in model organisms such as yeast, worms, flies and mice leading to lifespan extension suggest that longevity is subject to regulation. In addition, various system-wide interventions in old animals can reverse features of aging. To better understand these processes, much effort has been put into the study of aging on a molecular level. In particular, genome-wide microarray analysis of differently aged individual organisms or tissues has been used to track the global expression changes that occur during normal aging. Although these studies consistently implicate specific pathways in aging processes, there is little conservation between the individual genes that change. To circumvent this problem, we have recently developed a novel computational approach to discover transcription factors that may be responsible for driving global expression changes with age. We identified the transcription factor NFκB as a candidate activator of aging-related transcriptional changes in multiple human and mouse tissues. Genetic blockade of NFκB in the skin of chronologically aged mice reversed the global gene expression program and tissue characteristics to those of young mice, demonstrating for the first time that disruption of a single gene is sufficient to reverse features of aging, at least for the short-term.

The natural diversity in organismal lifespan suggests that longevity is subject to genetic regulation. Single gene mutations resulting in dramatic extensions of lifespan further bolsters the contention that aging is a genetically regulated process, rather than simply the result of organismal wear and tear over time. Understanding aging on a molecular level could thus provide us the ability to slow the aging process, prevent age-related disease and extend lifespan. Experiments in model organisms including yeast, worms, flies and mice reveal that several lifespan-related genes act through similar evolutionarily conserved pathways, the best characterized of which is the

insulin/insulin-like growth factor 1 (IGF-1) endocrine pathway.¹ For example, weak reduction-of-function mutations in *daf-2*, an insulin-IGF-1 receptor homologue, double the lifespan of *C. elegans* in a manner dependent on the *daf-16* transcription factor. In mice, the insulin-IGF-1 receptor has evolved into two separate receptors for insulin and IGF-1, and both receptors have been implicated in lifespan regulation: heterozygous mice containing half the normal levels of IGF-1 receptors live 30% longer than wildtype mice,² and knocking out the insulin receptor in adipose tissue of mice extends lifespan by 18%.³ Another highly conserved regulator of aging is the Sir2 family of NAD⁺-dependent lysine deacetylases that regulate gene activity and genomic integrity. Activation of Sir2 in several model organisms including yeast, worms and flies extends their lifespan.⁴ Further, there is growing evidence that mammalian Sir2 homologues (of which there are seven, SIRT1-7) may also have crucial roles in mammalian aging.⁵ These studies highlight the evolutionarily conserved genetic regulation of longevity. In a similar fashion, mutations in several genes involved in metabolism, stress responses and telomere regulation can also influence the lifespan of model organisms.¹

Besides extending the lifespan of an organism through genetic intervention, other studies have alternatively focused on methods to reverse the process of aging. Physiologic interventions such as caloric restriction, heterochronic parabiosis, ovarian transplant and exercise have all been shown to reverse aging phenotypes. Caloric restriction, defined as a 60 to 70% reduction in caloric intake, throughout the lifespan of organisms ranging from yeast to primates has repeatedly been shown to increase lifespan;⁶ reducing the caloric intake starting at an old age has also been shown to increase the lifespan of flies and mice, and this is sufficient to reverse gene expression changes associated with age in mice.⁷⁻⁹ These findings highlight the role of environmental factors such as diet in the aging process. Heterochronic parabiosis, or the sharing of circulatory systems, has also been shown to rejuvenate aged features in mice. Specifically, exposing an old mouse to a young systemic environment rejuvenated Notch signaling, cell proliferation and tissue regeneration capacity of aged muscle.¹⁰ Additionally, transplantation of young ovaries into middle-aged recipient females extends lifespan by 40% over intact females.¹¹ Lastly, recent work indicates that elderly humans can reverse age-induced gene expression changes associated with mitochondrial dysfunction in skeletal

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Submitted: 12/24/07; Accepted: 12/26/07

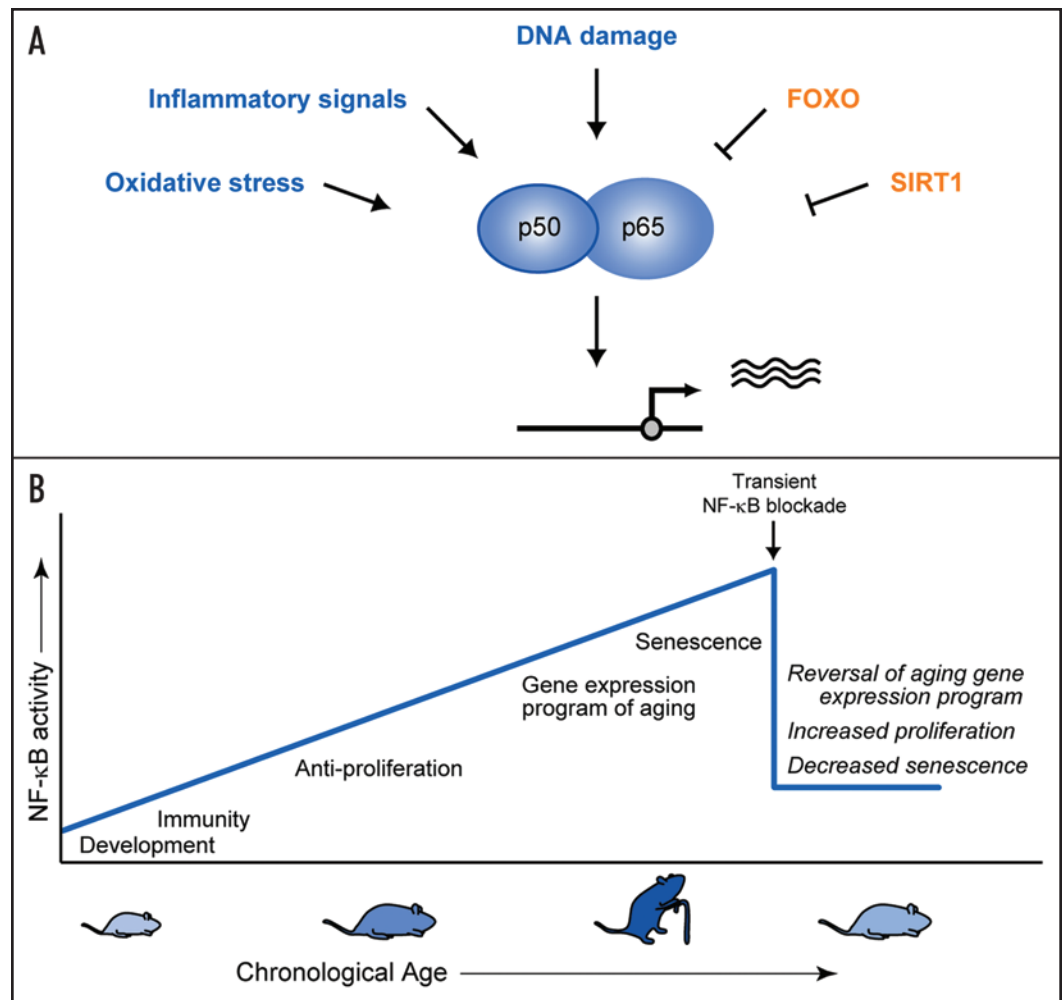
Previously published online as a *Cell Cycle* E-publication:
<http://www.landesbioscience.com/journals/cc/article/5490>

Figure 1. NF κ B action in aging. (A) NF κ B (shown as a p50–p65 heterodimer) is an ideal integrator of aging signals: NF κ B is activated by stressors that shorten lifespan (blue text), and NF κ B can be repressed by signals that extend lifespan (orange text). (B) Many age-specific functions of NF κ B are revealed as its activity accumulates with chronological age. Continual NF κ B activity appears to be required for age-associated phenotypes (in the skin), since transient blockade of NF κ B reverses multiple aging phenotypes to that of young.

muscle through resistance exercise training.¹² Together, these studies suggest that aging may be an active program that can be interrupted to retard the aging process. However, the molecular mechanisms by which these physiologic alterations influence the gene expression and phenotypes associated with aging still remain unclear.

To better understand the global changes that occur during aging and to further aid in elucidating the mechanisms of aging, many investigators have focused on characterizing the global gene expression changes that occur during aging. In particular, whole genome microarray analysis has been used to determine the expression changes that occur during normal chronological aging in individual organisms (such as *C. elegans* and *D. melanogaster*) or individual tissues of organisms (such as mouse and human).^{13–17} Many studies have focused on identifying the specific genes that change with age and whether these genetic changes are conserved in multiple tissues or organisms. While the expression of specific pathways has been found to consistently change with age, there is a lack of conservation between the individual genes changing with age, even between closely related tissues or species.^{14,16,17}

To address the challenge of identifying conserved regulators of gene expression changes with age, we recently developed a computational pipeline aimed at discovering candidate transcription factors responsible for driving these global expression changes.¹⁸ We first computationally identified the human target promoters of combinations of *cis*-regulatory motifs, which represent transcription factor binding sites. At the single promoter level, this task is difficult and the confidence in any one such prediction is limited. However, in subsequent analyses we jointly analyzed the motif targets as a set of genes, and this gene-set based analysis is the key to the robustness of our findings. Specifically, we next determined the expression levels of these motif targets with chronological age in six different human tissues. From this, we identified several candidate motifs whose gene targets showed a consistent increase or decrease in expression with age. We repeated the same analysis in several mouse tissues to identify



conserved candidate motifs that drive gene expression with age. The gene targets of one motif, corresponding to the binding site of the transcription factor NF κ B, stood out as showing a strong increase in expression with age in every human and mouse tissue analyzed; notably, NF κ B targets were also induced in cells isolated from prematurely aged humans, such as in the progeroid Hutchinson-Guilford syndrome.

NF κ B is a family of five transcription factors, characterized by the presence of an N-terminal Rel-homology domain necessary for dimerization and DNA binding.¹⁹ NF κ B functions as a dimer, the best studied of which is the heterodimeric complex of p50 (encoded by *NFKB1*) and p65 (encoded by *RELA*). In the inactive state, NF κ B complexes are sequestered in the cytoplasm by I κ B inhibitory proteins. Diverse signals such as the inflammatory cytokine TNF α , DNA damage, or oxidative stress stimulate phosphorylation and subsequent degradation of I κ B, leading to nuclear translocation of NF κ B. Notably, many of the classic activators of NF κ B are stressors that can accelerate organismal aging, making NF κ B an ideal integrator of aging signals in the body (Fig. 1A). Further, longevity signals such as SIRT1 and FOXO have been found to inhibit NF κ B activity, though the physiological relevance of these interactions has not been addressed (Fig. 1A).^{20,21} Within the nucleus, NF κ B dimers recognize and bind the consensus motif GGGRNYYCC to regulate the transcription of a wide variety of genes involved in innate and adaptive immunity, inflammation and apoptosis.^{19,22,23}

Previous studies have suggested that NF κ B may play a role in regulating aging. For instance, multiple groups have demonstrated that NF κ B DNA binding activity increases with age in rodent tissues.^{24,25} We too have verified that NF κ B DNA binding activity is induced with age in mouse skin, heart, kidney, liver and spleen.¹⁸ A recent study found evidence of increased NF κ B activation in aged murine hematopoietic stem cells.²⁶ And further, *in vitro* experiments have suggested that NF κ B regulates senescence, a form of cell cycle arrest commonly associated with aging: Hardy et al., found NF κ B motifs in promoters of genes induced in immortalized human fibroblasts following replicative senescence,²⁷ and Bernard et al. could induce senescence in primary human keratinocytes by *c-Rel* overexpression.²⁸ However, replicative and oncogene-induced senescence are not equivalent to aging, and the role of NF κ B in regulating *in vivo* senescence has not been analyzed. Thus we decided to determine if NF κ B is playing a direct role in aging, in particular aging-induced gene expression and cellular senescence.

Because NF κ B is important during animal development and *RelA*^{-/-} mice are embryonic lethal (Fig. 1B),^{19,29} it was not possible to determine if a NF κ B knockout animal could live longer. Instead, we focused on the tissue-specific role of increased NF κ B activity in an aged animal. In particular, we determined the role of NF κ B in aged mouse skin by utilizing a transgenic mouse system that allows inducible NF κ B blockade.³⁰ Dominant negative p50 protein was fused to a 4-hydroxytamoxifen (4-OHT)-responsive estrogen receptor (Δ SP-p50-ER) and expressed from a keratin 14 promoter, which drives expression in the basal layer of epidermal skin in a transgenic mouse. Consequently, the mutant protein normally remains inactive and localized in the cytoplasm; however, upon 4-OHT addition Δ SP-p50-ER translocates to the nucleus where it inhibits NF κ B transcriptional activity in the skin.^{18,30}

To study the role of NF κ B in regulating aged skin,¹⁸ we aged Δ SP-p50-ER transgenic mice 1.5–2 years in the absence of 4-OHT. The mice were then topically treated with 4-OHT (or ethanol as a control) for two weeks to block NF κ B activity specifically in the skin. Global gene expression profiling of the control-treated old skin compared to young skin treated in parallel revealed a set of >400 genes that were robustly induced with age. Surprisingly, upon 4-OHT treatment to inhibit NF κ B activity we found that more than half of these genes reversed their expression levels back to that observed in young mice. Consistently, unsupervised hierarchical clustering confirmed that the expression profile of NF κ B-blocked old skin was globally more similar to that of young skin than that of the control-treated skin from the same aged animals. We next analyzed the histology of the aged skin, which is normally characterized by atrophy and increased cellular senescence. NF κ B blockade increased the proliferative capacity of the skin and reversed several markers of cellular senescence to levels observed in young animals. Lastly, we determined that the role of NF κ B in regulating the aging gene expression program was age-specific, since inhibiting NF κ B in young transgenic mice could induce proliferation but had no effect on the gene expression signature of aging. Together, these results demonstrate that NF κ B activity is continually required to maintain the global gene expression program and cellular senescence associated with age in mouse skin (Fig. 1B).

Our study showed that many molecular features of mammalian aging may be reversed by the focal genetic blockade of NF κ B,

revealing a surprising degree of plasticity of aging. The potential significance of reversal of aging is three-fold. First, from a mechanistic perspective, the reversal of aging by a genetic intervention applied very late in life suggests that aging is an active genetic program that must be continually reinforced to prevent a default state of being young, at least in skin. In other words, in addition to genes that control the rate of aging that act throughout the lifetime of the animal, there are other genes that must act specifically in old cells to keep them old. This concept complements the existing data indicating that aging also involves passive accumulation of stochastic wear and tear.^{31,32} Second, reversal of aging by a genetic intervention implies an indispensable and dominant role of NF κ B in the aging program. While many pathways, including p53, DNA damage checkpoints, telomere shortening, and oxidative damage, have been postulated to contribute to senescence and *in vivo* aging,³³ whether reversal of mammalian aging is possible by blockade of any of these pathways had not been shown. Our unbiased genome-wide screen of gene expression in aged human tissues supports a uniquely dominant role of NF κ B in human aging. Even when myriad pathways are activated by stress or other insults in chronologically aged tissue, NF κ B appears to be an essential mediator that is continually required to enforce phenotypes associated with aging. Third, the reversal of aging has important practical and medical implications. With knowledge of causality of aging, one would expect that prevention of these causes—over the lifetime of a young individual—might delay the rate of aging. In contrast, evidence of reversibility of aging predicts that one may treat age-associated pathologies in already elderly individuals by therapies applied late in life.

At present, it is unclear whether NF κ B blockade can become the much sought-after “fountain of youth”. The reversal of age-related phenotypes in mouse skin was only short-term; thus we do not know whether long-term reversal of aging is possible or whether tissue longevity has been extended. Further, the consequences of long-term blockade is not known, and inactivation of cell senescence by inhibiting NF κ B may permit cancerous growth in the presence of additional oncogenes.³⁴ Consequently, therapeutic roles of NF κ B blockade in aging may involve temporary rejuvenation of aged skin or other tissues to allow quicker healing following injury. NF κ B blockade may also alleviate several age-related diseases, including muscle atrophy, insulin resistance and neurotoxicity in Alzheimer’s disease,^{35–37} without permanent rejuvenation. Addressing these topics, along with further elucidating the exact mechanism by which NF κ B regulates aging, will be important issues for future study.

Acknowledgements

Supported by grants from the National Institutes of Health, American Cancer Society, the California Breast Cancer Research Program, and the Damon Runyon Cancer Research Foundation.

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