

WORKSHOP
**Cell Autonomous and Non-Cell Autonomous Mechanisms
of Aging**

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Cell Autonomous and Non-Cell Autonomous Mechanisms of Aging

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Aging is arguably the most important, yet poorly understood aspect of biology. There is compelling evidence to support the hypothesis that the underlying cause of aging is the cell autonomous, time-dependent accumulation of stochastic damage to cells, organelles, and macromolecules, including DNA damage (**Figure 1**). It is also clear from heterochronic parabiosis, serum transfer, and cell ablation studies that cell non-autonomous mechanisms play important roles in driving degenerative changes that arise as the consequence of spontaneous,

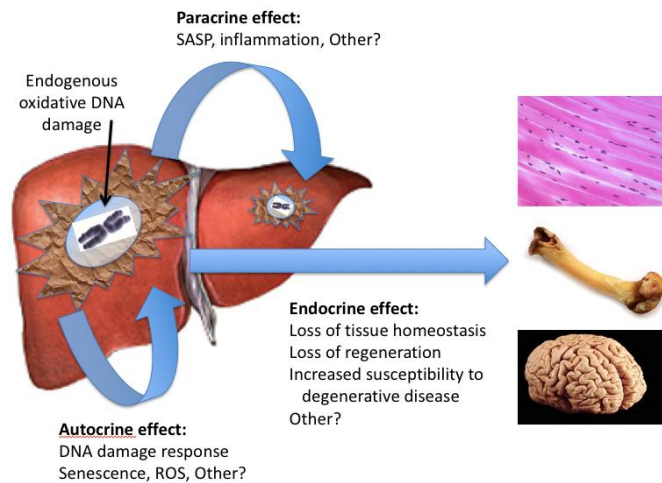


Figure 1. Cell autonomous and non-autonomous pathways of aging. Damage to cells (e.g., DNA damage) can cause cell autonomous effects including apoptosis or senescence driven by altered mitochondrial function or increased ROS. The damaged cell also can affect adjacent cells (paracrine effects) as well as cells at a distance (endocrine effects) to drive aging through release of pro-geronic factors such as factors secreted by senescent cells.

contribute to aging. The long term goal will be to identify drugs molecular targets in these pathways that will lead to therapeutic approaches to extend healthy aging as well as delay aging-related diseases such as osteoarthritis (OA).

stochastic damage (**Figure 1**). However, the relative contribution of cell autonomous and non-autonomous mechanisms to systemic aging in different organisms is unclear. We employ mice with genetically perturbed DNA repair in order to accelerate the accumulation of spontaneous stochastic damage to DNA *in vivo*. *Erc1*^{-Δ} mice have reduced expression of the DNA repair enzyme ERCC1-XPF. These mice spontaneously accumulate endogenous DNA damage faster than wild-type mice with normal DNA repair. This causes the mice to age 6X faster than normal mice and to spontaneously develop numerous age-related diseases within their 7 month lifespan, including osteoporosis, disc degeneration and impaired muscle regeneration. To investigate the relative importance of cell autonomous and non-autonomous mechanisms of aging, we also deleted ERCC1-XPF tissue-specifically and measured the effect on the targeted tissue as well as non-targeted tissues. These new mouse models of tissue- and cell type-specific aging now can be used to assess pathways important for driving local and systemic aging, loss of tissue homeostasis and regeneration, and stem cell dysfunction. The goal of this workshop is to educate and inform the ORS members about cell autonomous and non-autonomous mechanisms that

Identification and characterization of key cell and tissue types that drive aging

Laura Niedernhofer, MD, PhD, The Scripps Research Institute

There is general agreement that cell autonomous mechanisms contribute to aging. As cells accrue damage over time, they respond by triggering individual cell “fate” decisions (e.g., senescence and apoptosis) that ultimately disrupt tissue homeostasis and thereby increase risk of morbidity. To investigate these mechanisms, we genetically depleted the nuclear DNA repair enzyme ERCC1-XPF in mice allowing us to focus on the impact of stochastic DNA damage that occurs because of normal metabolic

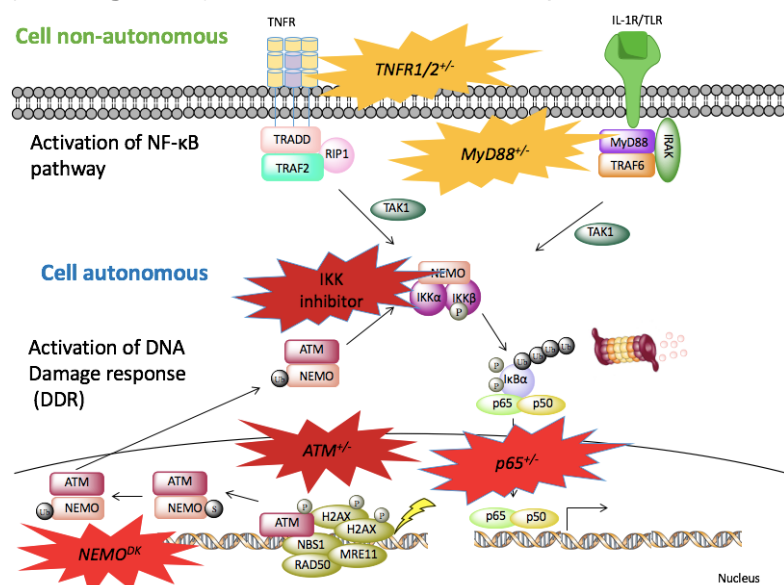
Table 1. Tissue-specific deletion of *Erc1* and its consequences.

Promoter	Target cell	Effect on targeted tissue	Non-targeted tissue affected?
CamKIIα	neurons CNS	cerebral atrophy, cognitive decline	none
Alb	hepatocytes	none detected	none
Ckmm	myocytes	congestive heart failure	lung
Pod	podocytes	chronic kidney disease	?
Rip	β -cells	adult onset diabetes	liver, adipose
Pax6	lens epithelia	cataracts	none
HS21/45 (Vav)	hematopoietic	immune dysfunction	liver, kidney, lung, aorta, brain, spleen

processes. ERCC1-deficient mice accumulate oxidative DNA damage more rapidly than wild-type animals that are able to repair their DNA. The *Ercc1* mutants also accumulate senescent cells more rapidly than wild-type mice. Unexpectedly, we observed that the increased DNA damage triggers mitochondrial and metabolic changes, as well as *increased* oxidant production, illuminating a cell autonomous mechanism of damage amplification. We also genetically depleted ERCC1-XPF in specific cell types of mice using Cre-Lox technology (**Table 1**). This revealed marked differences in how the targeted cells responded to increased stochastic damage, ranging from no apparent malfunction to senescence or premature cell death. In these models, there were also marked differences in how the non-deleted tissues responded. For example, knock-out of *Ercc1* in the hematopoietic system triggered a significant increase in expression of senescence markers and loss of homeostasis in several tissues, whereas knock-out in differentiated myocytes only affected the heart. These results suggest that aging of specific cell types drives systemic aging and thus represent possible therapeutic targets.

Identification of key cell autonomous and non-autonomous signaling mechanisms involved in aging
 Paul Robbins, PhD, The Scripps Research Institute

A key mediator of the cellular response to genotoxic, oxidative and inflammatory stress is the transcription factor NF-κB. NF-κB is activated with aging and in numerous aging-related degenerative diseases. We have examined the effect of stochastic DNA damage in driving NF-κB activation in a cell autonomous or non-autonomous manner (see **Figure 2**) and determined the impact this has on neighboring cells and distant tissues. We have



demonstrated that NF-κB is activated in a stochastic manner with accelerated and natural aging in a variety of cell types including in CD31⁺ cells, CD11b⁺ cells (MDSCs) in bone marrow and spleen, kidney podocytes and multiple cell types in the liver, intestine and pancreas. We also demonstrated that non-autonomous NF-κB activation via TNF-Rs and IL-1R/TLR has a more minor effect on overall NF-κB activation and healthspan, affecting the pathology of only specific cells (e.g. muscle stem cells) or tissues (e.g. intervertebral discs, bone) non-autonomously. However, reducing or inhibiting NF-κB activation genetically or pharmacologically had a significant effect on reducing cell senescence, improving endogenous stem function and extending healthspan. Moreover, we demonstrated that elimination of senescent cells that arise with aging, in part through NF-κB activation, using senolytic drugs (e.g. drugs that kill senescent cells specifically) extends healthspan in mouse models of accelerated aging.

Figure 2. Genetic analysis of pathways activating NF-κB, either cell autonomously or non-autonomously. Genes highlighted in red (highly significant) or orange (less significant) are pathways shown to affect NF-κB activation, senescence and aging.

This extension of healthspan includes a reduction in osteoporosis and disc degeneration. These results demonstrate that reducing the extent of activation of specific pathways such as IKK/NF-κB in response to stochastic damage can reduce senescence, inflammation and aging.

Effect of tissue and cell type-specific aging on stem cell and stem cell niche function

Johnny Huard, PhD, The University of Texas Health Science Center at Houston

A universal characteristic of aging is loss of tissue regenerative potential, which leads to an impaired ability to respond to stress and, as a consequence, dramatically increases the risk of morbidity and mortality. This and

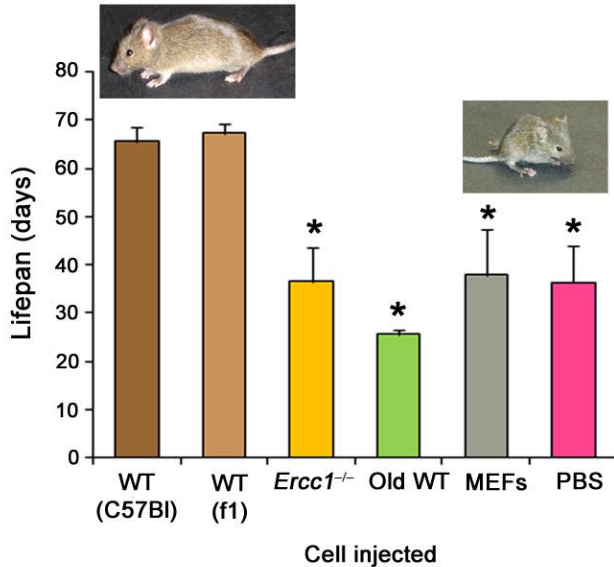


Figure 3. Transplantation of young stem cells extends lifespan and healthspan. IP injection of muscle-derived stem/progenitor cells from young mice extends the lifespan of *Ercc1*-deficient mice. In contrast, injection of MDSPCs from old mice reduces lifespan.

the exponentially increased incidence of numerous degenerative diseases in the elderly has led to the hypothesis that aging is caused, in part, by the loss of functional stem cells necessary for maintaining tissue homeostasis. However, what drives age-associated stem cell loss and dysfunction, and the effects on aging have not been clearly defined. It also remains unclear if the defects in stem cell function arise due to defects in the stem cells (cell autonomous) or in the stem cell niche (non-autonomous). We demonstrated that function of muscle-derived stem/progenitor cells (MDSPC) and bone marrow-derived mesenchymal stem cells (MSCs) declines with natural and accelerated aging. We also demonstrated that aging of specific cell types or tissues, including β cells and endothelial cells in mice results in MDSPC dysfunction through a cell non-autonomous pathway(s). Finally, we demonstrated that injection of as little as 10^6 MDSPCs or MSCs can extend healthspan in two different murine models of accelerated aging (Figure 3). At least part of this effect is mediated through release of extracellular vesicles (EVs) from MDSPCs and MSCs. These results provide strong support for the hypothesis that paracrine/endocrine effects imparted by functional progenitor cells on the host tissue

microenvironment play a major role in tissue repair and in maintaining tissue homeostasis. These results also demonstrate that stem dysfunction is driven through both cell autonomous and non-autonomous pathways.