Published in final edited form as:

Pharmacol Res. 2016 September ; 111: 152–154. doi:10.1016/j.phrs.2016.05.015.

Perspective: Targeting the JAK/STAT pathway to fight age-related dysfunction

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Abstract

Senescent cells accumulate in a variety of tissues with aging. They can develop a senescence-associated secretory phenotype (SASP) that entails secretion of inflammatory cytokines, chemokines, proteases, and growth factors. These SASP components can alter the microenvironment within tissues and affect the function of neighboring cells, which can eventually lead to local and systemic dysfunction. The JAK pathway is more highly activated in senescent than non-senescent cells. Inhibition of the JAK pathway suppresses the SASP in senescent cells and alleviates age-related tissue dysfunction. Targeting senescent cells could be a promising way to improve healthspan in aged population.

Abstract

Aging is one of the leading risk factors for a variety of age-related diseases, including atherosclerosis, most cancers, diabetes, osteoporosis, neurodegeneration, and frailty (1). The precise mechanisms through which aging predisposes to these disorders are still unclear. Cellular senescence refers to a state of irreversible proliferative arrest in replication-competent cells (2) and has been associated with many age-related diseases (3–5). With aging, senescent cells accumulate in multiple tissues (6–12) and secrete a wide range of pro-inflammatory cytokines, chemokines, and growth factors, termed the senescence-associated secretory phenotype (SASP) (13, 14). Emerging evidence suggests that eliminating senescent cells *in vivo* can slow down manifestations of the aging process and alleviate age-related tissue dysfunction in old mice (15–17). These exciting studies point to a causal role for senescent cells in age-related dysfunction and diseases, indicating that senolytic drugs,

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which selectively kill senescent cells, might be an effective means for increasing healthspan and improving function in old age.

It is still not clear why killing a relatively small number of senescent cells can improve function of a range of tissues. Senescent cells appear to be able to spread senescence to nearby cells (18, 19). In addition, we recently published two studies (20, 21) proposing that specific SASP components secreted by senescent cells can exert bystander effects on nearby healthy cells, leading to dysfunction. We isolated primary fat progenitor cells (preadipocytes or adipose-derived stem cells) from the adipose tissue of healthy human subjects. We then made them senescent using either radiation or serial passaging. To test for bystander effects, we collected conditioned media (CM) from these senescent cells. We found that CM from senescent cells induced macrophage migration and increased inflammatory gene expression in human adipose tissue and preadipocytes. The CM also impaired adipogenesis: healthy preadipocytes were less able to differentiate into fat cells. These pieces of evidence indicate that senescent cells contribute to age-related chronic inflammation and impaired function of adipose tissue in a paracrine manner.

Prompted by these deleterious effects of the SASP *in vitro*, we tested if it is feasible to alleviate age-related dysfunction by inhibiting the SASP in old animals. The JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway is one of the major mechanisms that regulates cytokine production (22, 23). Thus, this pathway has potential as a therapeutic target. The JAK family consists of 4 members, including JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2). These four members act through downstream STAT proteins to control a variety of biological events, such as immune regulation and hematopoiesis. JAK1 and 2 play essential roles in inflammatory signaling and in regulating growth hormone and other endocrine signals (24). JAK3 is principally expressed in cells of the hematopoietic lineage and is involved in erythropoietin signaling and immune function (25, 26). TYK2 plays a critical role in immune cell function and host defenses (27, 28). Based on these various roles, JAK pathways have been investigated as therapeutic targets for a number of diseases. Ruxolitinib is a specific JAK1/2 inhibitor that has been approved by FDA for treating myelofibrosis, a condition associated with a have gain-of-function JAK2 mutation in a substantial proportion of patients (29). Tofacitinib is a JAK1/3 inhibitor that has been approved by the FDA for treating rheumatoid arthritis. Other inhibitors that target different JAKs are in clinical trials for treating myelofibrosis (22, 30), acute myeloid leukemia (31), lymphoma (32), and rheumatoid arthritis (22, 33, 34). Furthermore, JAK inhibitors have been found to improve antitumor responses by reprogramming the SASP in senescent cells (NVP-BSK805, JAK2 inhibitor) (35), promoting hair growth (ruxolitinib and tofacitinib) (36), and improving muscle stem cell function and muscle regeneration (Tyrphostin AG 490, JAK2 inhibitor) (37).

In our studies, the JAK pathway was more highly activated in fat tissue from old than young animals and senescent than non-senescent cells. Inhibition of the JAK pathway in senescent cells by treating with JAK inhibitors or siRNA's suppressed the SASP (21). Notably, inhibition of the JAK pathway attenuated the SASP in two human cell types of distinct lineages: primary human preadipocytes and human endothelial cells (21). This suggests that the JAK pathway might drive the SASP in multiple cell types. We found that 2 months of

ruxolitinib administration reduced systemic inflammation, enhanced physical capacity, preserved fat tissue homeostasis, and improved metabolic function in 22–24 month old mice (20, 21). Interestingly, most of these beneficial effects were not obvious, if they occur at all, in 7 month old mice given the same treatment. This finding that inhibition of the JAK1/2 pathway had beneficial effects in older but not younger individuals suggests that fundamental aging processes may have been targeted, such as age-related senescent cell accumulation.

It is quite likely that effects on cells other than senescent cells also contributed to the beneficial effects of JAK inhibition. Due to the role of JAK pathways in immune responses, we tested the effect of the JAK1/2 inhibitor on peripheral blood immune cells in aged mice and found no substantial change in either young or aged mice, which is consistent with previous findings (38). Also, ruxolitinib has much less effect on JAK3 and TYK2, the JAK family members most implicated in regulating erythropoiesis and immune function, than JAK1 and 2 (38). Furthermore, genetic or pharmacological senescent cell clearance in progeroid mice (15) or naturally aged mice (17) alleviated frailty. We found that genetic clearance of senescent cells improved adipogenesis in fat tissue in older, naturally-aged mice (20), a finding that was subsequently confirmed by others (39). All these interventions specifically targeting senescent cells had effects similar to those we found using pharmacological inhibition of JAK1/2. Moreover, the JAK1/2 inhibitor had beneficial effects principally in aged mice, which have more senescent cells than young mice. Together, these findings are consistent with the possibility that JAK inhibitors have beneficial effects through an impact on senescent cells.

In addition to JAK inhibitors, other compounds have been noted to suppress SASP components, including metformin (40), rapamycin (41), and glucocorticoids (42). Rapamycin is an mTOR pathway inhibitor. It was shown to extend lifespan in mice (43), while it had limited effects on age-related tissue dysfunction (44). Metformin is a commonly used drug for treating type 2 diabetes. It has been shown to improve physical function, increase insulin sensitivity, and reduce lipotoxicity in aged mice (45), effects similar to those of JAK inhibitors. Both rapamycin and metformin treatments were initiated during middle age and continued for at least 24 weeks. In comparison, we treated already aged mice (22–24 months old) with a JAK inhibitor for 10 weeks or less, yet we observed reduced inflammation and improved physical function, including grip strength, endurance, and physical activity. This suggests that it may be possible to alleviate frailty in older individuals even with relatively short-term treatment. We hypothesize that it might also be feasible to treat elderly people to achieve temporary physical improvement before or after such medical interventions as surgery or chemotherapy. Indeed, ruxolitinib has been shown to increase activity and reduce frailty-like symptoms in elderly human subjects (median age of 66) with a cancer-like disease in a recent clinical trial (46). In addition, since frailty is highly associated with inflammation (47), we speculate that JAK inhibitor treatment might be more effective in patients with a high level of inflammation. This means that testing to predict who may benefit most from this intervention for frailty might be feasible. Potential candidates for such a test may be circulating inflammatory cytokines, such as IL-6 or TNF- α , or markers of generalized inflammation, such as C-reactive protein. These speculations need to be investigated further and tested in clinical trials before use in patients.

JAK inhibitors can have side effects, including anemia and thrombocytopenia, at least in human subjects with myelofibrosis (46, 48). Although no severe side-effects were observed in our study, the fact that JAK inhibitors may have to be administered continuously rather than intermittently to suppress the SASP increases the likelihood of side-effects. Effort is needed to check for potential side-effects in pre-clinical studies and eventually in clinical trials in older subjects and also to determine the optimal duration of JAK inhibitor administration for patient safety. Senolytic drugs offer an alternative option, since it may be feasible to administer them intermittently instead of continuously (49). However, senescent cells appear to be important for several biological processes, including prevention of fibrosis (3), promotion of wound healing (50), and insulin secretion (51). Therefore, eliminating senescent cells might also have side-effects. Another strategy could be to inhibit specific SASP components relevant to particular pathologies, such as activin A in the case of adipose tissue dysfunction or IL-6 for frailty. All these potential approaches merit further investigation.

In summary, mounting evidence indicates that targeting senescent cells could be a very promising way to enhance healthspan and delay, prevent, alleviate, or treat age-related chronic diseases. Furthermore, this strategy could be effective in older, already disabled individuals, over and above use for preventive applications (21). However, we are just at the beginning of a very long road toward devising interventions to improve healthspan in older people. More mechanistic understanding of the biology of aging, and particularly about cellular senescence, is required. Several major concerns remain to be addressed, including potential side effects and optimal drug administration paradigms before we can move these treatments into clinical use.

Acknowledgments

The authors are grateful for financial support from the NIH (grants AG013925, AG044396, and AG19899), the Robert and Arlene Kogod Center on Aging, the Connor Group, and the Ted Nash Foundation. M.X. received a Glenn/American Federation for Aging Research Postdoctoral Fellowship for Translational Research on Aging.

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