Experimental Gerontology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Clinical strategies and animal models for developing senolytic agents

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ARTICLE INFO

Article history:
Received 3 October 2014
Received in revised form 20 October 2014
Accepted 26 October 2014
Available online xxxx

Section Editor: Kurt Borg

Keywords:
Cellular senescence
Senolytic
Senescence-associated secretory phenotype
Healthspan

ABSTRACT

Aging is associated with increasing predisposition to multiple chronic diseases. One fundamental aging process that is often operative at sites of the pathology underlying chronic age-related diseases is cellular senescence. Small molecule senolytic agents are being developed. For successful drug development: 1) appropriate animal models of human age-related diseases need to be devised. 2) Models have to be made in which it can be proven that beneficial phenotypic effects are actually caused through clearing senescent cells by putative senolytic agents, as opposed to "off-target" effects of these agents on non-senescent cells. 3) Models are needed to test efficacy of drugs and to uncover potential side effects of senolytic agents. Development of the optimal animal models and clinical trial paradigms for senolytic agents warrants an intensive effort, since senolytic agents, if successful in delaying, preventing, alleviating, or reversing age-related diseases as a group would be transformative.

1. Introduction

Aging is associated with increasing predisposition to multiple chronic diseases: atherosclerosis, cancers, dementias, diabetes, arthritis, and many others (Goldman et al., 2013; Kirkland, 2013a,b; Research, 2012). Chronological age is the biggest risk factor for many of these diseases and in some cases is a better predictor than all other known risk factors combined. One fundamental aging process that is often operative at sites of pathology underlying chronic age-related diseases is cellular senescence (Kirkland, 2013a; Tchkonia et al., 2013).

Cellular senescence refers to the essentially irreversible cell cycle arrest caused by potentially oncogenic and metabolic insults (Tchkonia et al., 2013). Senescent cells can acquire a senescence-associated secretory phenotype (SASP) that involves release of pro-inflammatory cytokines, chemokines, pro-thrombotic factors, and extracellular matrix proteases that cause tissue damage, as well as extracellular matrix proteins that can contribute to dysfunctional tissue architecture or fibrosis. Thus, the adverse pathogenic mechanisms at the tissue level that could be promoted by cellular senescence include chronic inflammation, loss of functional progenitor cells, clotting, and extracellular matrix dysfunction.

Senescent cells accumulate in multiple tissues with advancing age (Tchkonia et al., 2013; Waaijer et al., 2012). Senescent cell burden is, in turn, associated with lifespan. At 18 months of age, long-lived Ames dwarf, Snell dwarf, and growth hormone receptor knockout (GHRKO) mice have fewer senescent cells in their fat tissue than age-matched control wild-type animals, while short lived growth hormone overexpressing mice have more (Stout et al., 2014). Caloric restriction sufficient to increase lifespan in mice is associated with decreased expression of $p16^{Ink4a}$, a senescence marker, in multiple tissues compared to *ad libitum*-fed animals (Krishnamurthy et al., 2004). Progeroid mice, including mouse models of Werner and Hutchinson–Guilford progerias, as well as *Klotho*-deficient, $Ercc^{-/-}$, and $BubR1^{H/H}$ mice have increased senescent cells (Baker et al., 2008; Chen et al., 2013; Eren et al., 2014b; Tchkonia et al., 2013). In comparisons across longer- *vs.* short-lived mouse cohorts, senescent cell accumulation in liver and intestinal crypts predicts mean and maximum lifespan (Jurk et al., 2014). Cellular senescence can occur at any point during life, even in blastocysts (Meuter et al., 2014) and in the placenta (Rajagopalan and Long, 2012). Indeed, senescence is important in remodeling during embryogenesis (Munoz-Espin et al., 2013; Storer et al., 2013).

These associations between cellular senescence, aging, and age-related pathologies prompted testing if senescent cell clearance ameliorates dysfunction. Genetically targeting senescent cells in *INK-ATTAC; $BubR1^{H/H}$* progeroid mice that express a drug-activatable "suicide" gene only in senescent cells enhanced healthspan (Baker et al., 2011), the portion of the lifespan during which freedom from pain, disability, and dependence is enjoyed (Kirkland and Peterson, 2009). Even clearing only around 30% of senescent cells from these mice led to partial reversal of age-related lipodystrophy and decreased progression of frailty,

Abbreviations: SA-βgal, senescence-associated β-galactosidase; SASP, senescence-associated secretory phenotype; FDA, Food and Drug Administration; GHRKO, growth hormone receptor knockout; ATTAC, apoptosis through targeted activation of caspase 8; BubR1, budding uninhibited by benzimidazoles-1; PAI-1, plasminogen activator inhibitor-1; MMP, matrix metalloproteinase.

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http://dx.doi.org/10.1016/j.exger.2014.10.012
0531-5565/

sarcopenia, and cataract formation (Baker et al., 2011; Tchkonia et al., 2013).

These findings have spurred development of small molecule senolytic agents and other approaches to decrease senescent cell burden, including peptides, RNA interference, and vaccines. For this effort to succeed: 1) appropriate animal models of human age-related diseases need to be developed. 2) Models have to be made to prove that beneficial effects are actually caused through clearing senescent cells by putative senolytic agents. Without this proof, it would be possible that the candidate agent leads to senescent cell clearance, but that phenotypic improvement is due to "off-target" effects on non-senescent cells, not directly through senescent cell clearance. 3) Models are needed in which possible side effects of senolytic agents can be tested. It should be noted that even though continual genetic clearance of senescent cells from mice did not lead to any overt side effects during 20 months of observation (Baker et al., 2011), there is evidence that cellular senescence has beneficial effects under some circumstances. For example, cellular senescence protects against cancer development, helps to resolve tissue fibrosis during healing, is involved in immune responses, and can contribute to tissue remodeling (Krizhanovsky et al., 2008; Tchkonia et al., 2013; Xue et al., 2007).

2. Associations between diseases in humans and cellular senescence

Cellular senescence is associated with many of the chronic diseases and disabilities that are the leading drivers of morbidity, mortality, and health costs (Kirkland, 2013a; Tchkonia et al., 2013; Zhu et al., 2014). Senescent cells have been identified at sites of pathology in a number of these conditions and may have systemic effects that predispose to others. These include: 1) metabolic conditions (diabetes, obesity, metabolic syndrome, and age-related lipodystrophy (Minamino et al., 2009; Tchkonia et al., 2010)), 2) cardiovascular disorders (atherosclerosis, hypertension, heart failure, and peripheral vascular disease (Holdt et al., 2011; Kirkland, 2013a; Minamino et al., 2002; Wang and Bennett, 2012; Westhoff et al., 2008)), 3) frailty (sarcopenia (Baker et al., 2011; Tchkonia et al., 2013)), 4) blindness (cataracts, glaucoma, macular degeneration (Baker et al., 2011; Kozlowski, 2012; Liton et al., 2005)), 5) loss of resilience (side effects shortly after or many years after chemotherapy or radiation, delayed recovery after elective surgery or acute events such as myocardial infarction (Kirkland, 2013a; Le et al., 2010; Marcoux et al., 2013; Roninson, 2003; Tchkonia et al., 2013)), 6) neurodegenerative diseases (Alzheimer's disease and "tau-opathies", Parkinson's, "chemo brain" after, for example, cis-platinum, HIV dementia (Chinta et al., 2013; Golde and Miller, 2009; Kirkland, 2013a; Krull et al., 2013)), 7) bone disorders (osteoporosis, osteoarthritis, fracture non-union (Bajada et al., 2009; Chen et al., 2013; Freund et al., 2010; Price et al., 2002)), 8) lung conditions (idiopathic pulmonary fibrosis, bleomycin lung and other drug- or environmental-toxin related lung diseases, and chronic obstructive lung disease (Aoshiba and Nagai, 2009; Barnes, 2013; Minagawa et al., 2011; Tsuji et al., 2004, 2009)), 9) liver disease (primary biliary cirrhosis (Tabibian et al., 2014), 10) genitourinary dysfunction (age-related glomerulosclerosis, predisposition to acute tubular necrosis, diabetic renal disease, prostatic hypertrophy (Castro et al., 2003; Choi et al., 2000; Clements et al., 2013; Kirkland, 2013a; Kitada et al., 2014)), 11) skin disorders: melanocytic naevi, chronic skin ulcers (bedsores) (Gray-Schopfer et al., 2006; Vande Berg et al., 2005), 12) cancers (Campisi and d'Adda di Fagagna, 2007; Kirkland, 2013a; Liu and Hornsby, 2007), 13) toxin exposures and drug or radiation treatments (drugs: alkylating and other chemotherapeutic agents (Roninson, 2003), HIV protease inhibitors (Torres and Lewis, 2014), hormones: long term growth hormone treatment (Stout et al., 2014), toxins (Welford et al., 2010), long term effects of therapeutic or accidental radiation (Marcoux et al., 2013)), 14) genetic disorders (such as progerias (Benson et al., 2010)), 15) infections (notably, HIV (Torres and Lewis, 2014)), and 16) chronological aging itself (Tchkonia et al., 2013; Waaijer et al., 2012). Some of these conditions will likely be the subjects of proof-of-concept clinical studies with senolytics to test whether clearing senescent cells provides clinical benefit.

3. Potential scenarios for initial proof-of-concept studies of senolytic agents

Initial clinical studies of senolytic agents will most likely involve indications in which short term administration leads to measurable clinical benefits in already symptomatic subjects, rather than studies of lifespan or healthspan. The first clinical studies may need to be publicly funded in academic settings if they involve repurposed agents that are off-patent. Existing agents still under patent, patentable methods of administration (*e.g.*, aerosol, topically, or ophthalmic drops), novel drug combinations, or new chemical entities will be more attractive to the pharmaceutical industry. The time to get new chemical entities to the point of proof-of-principle studies will be longer than for approved, repurposed agents. However, companies with approved agents under patent may be willing to explore new indications.

Animal models are needed that reflect the designs of initial human studies in order to facilitate preclinical testing of candidate senolytic agents. The first human studies may be small proof-of-principle trials of repurposed agents already approved for other applications that turn out to have senolytic activity. If this assumption is correct, possible scenarios in which such agents might first be tested include: A) amelioration of multiple co-morbidities, frailty, or loss of resilience, B) accelerated aging-like conditions, C) otherwise fatal conditions associated with cellular senescence, and D) localized conditions associated with senescence.

3.1. Amelioration of multiple co-morbidities, frailty, and loss of resilience

Fundamental aging mechanisms, including cellular senescence, are associated with multiple age-related chronic diseases. These diseases frequently occur within the same older individuals. Therefore, senolytic or SASP-protective agents may simultaneously ameliorate recognized short-term problems related to several different diseases within older subjects with multiple co-morbidities. Testing this will require novel clinical study paradigms. To date, clinical trials have focused on younger subjects with a single target condition, excluding older subjects with co-morbidities. A potential scenario for initial small-scale proof-of-principle trials of candidate senolytic or SASP-protective drugs would be to study their effect on multiple endpoints in elderly subjects with combinations of 2 or more of: atherosclerosis, hypertension, memory impairment, diabetes, COPD, renal dysfunction, or other cellular senescence-related conditions. These endpoints could be surrogate endpoints already recognized by regulatory agencies, such as circulating lipids, left ventricular function or hypertrophy, blood pressure, memory tests, fasting glucose or HOMA, and pulmonary function tests. To increase statistical power and reduce the number of subjects required, the endpoints could be combined into a composite score, although this approach carries the risk that an effective drug may seem less than effective if one of the composite endpoint components is affected in a direction opposite to that expected. For example, rapamycin may lead to improvements in a number of age-related measures of function, but also results in decreased glucose tolerance (Lamming et al., 2012).

Another scenario to test senolytics would be to ascertain if they ameliorate frailty. Frailty is an age-related syndrome that entails loss of resilience, with failure to respond well to, or recover from, acute challenges, such as chemotherapy, surgery, pneumonia, stroke, influenza, heart attacks, dehydration, immunization, or fractures (Bandeen-Roche et al., 2006, 2009; Fried et al., 2001; Kanapuru and Ershler, 2009; Kirkland, 2013b; Leng et al., 2007; Qu et al., 2009; Rockwood et al., 2006; Walston et al., 2002, 2006, 2009). Frailty can be diagnosed using scales that are reasonably, but not completely, sensitive and specific. These involve combinations of assessments of muscle weakness and sarcopenia, fatigue, weight loss, low activity, and chronic disease and disability

Please cite this article as: Kirkland, J.L., Tchkonia, T., Clinical strategies and animal models for developing senolytic agents, Exp. Gerontol. (2014), http://dx.doi.org/10.1016/j.exger.2014.10.012