INTRODUCTION

Aging, especially when coupled with unhealthy lifestyles, is the leading contributor to most physical dysfunctions and chronic diseases in humans, whereby senescent cells defined as division-arrested normal cells progressively accumulate in tissues (Childs et al., 2015; Collado et al., 2007). While gradual cellular senescence throughout the whole aging process was identified by Hayflick and Moorhead in 1961, to date, rare anti-aging and age-associated disease medicine has been developed targeting these biological aging processes at the cellular level. Moreover, unhealthy lifestyles, such as physical inactivity, unhealthy diets, and cigarette smoking, have been found to be associated with the accumulation of senescent cells in humans independent of chronological age (Liu et al., 2009; Song et al., 2010; Tchkonia et al., 2010). Importantly, cellular senescence also participates in other vital biological processes, not only aging but tumor...
suppression, tumor promotion, and tissue repair (Rodier & Campisi, 2011). Therefore, while cellular senescence is a promising therapeutic target of multiple diseases, especially aging-related diseases and cancer, the consequences and safety of up- or downregulating these complex biological processes should be taken into consideration.

Senescent cells are the hallmark and therapeutic target of cellular senescence involved in a wide range of biological processes, including tumor suppression, embryonic development, wound healing and tissue repair, and aging (Van Deursen, 2014). Although senescent cells are detrimental to the body during aging and can lead to chronic diseases, such as obesity, diabetes, and sarcopenia, they also suppress cancer and fibrosis (Muñoz-Espín & Serrano, 2014).

Under the complex functions, there are two major senescent pathways: p53/p21Cip1 and p16INK4a/RB; and both p21Cip1 and p16INK4a are the key markers of senescent cells (Ben-Porath & Weinberg, 2005). Moreover, other markers of senescent cells have been reported, including senescence-associated heterochromatic foci (SAHF’s), ARF, DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS), senescence-associated beta-galactosidase (SA-β-Gal), shorter telomere length, lower proliferation, and senescence-associated secretory phenotype (SASP) (He & Sharpless, 2017). Exploration of these senescent markers as targets might contribute to the development and testing of anti-senescent cell therapeutic strategies.

Senolitics is a new class of medicines that target senescent cells, which has emerged rapidly in the past few years (Kirkland et al., 2017). Preclinical studies on rodents have been applied to explore the potential targets of senescent cells and the preliminary effects of senolytic medicine in vivo. In 2011, an INK-ATTAC transgenic mouse with a BubR1 progeroid background was first established and showed restored aging-associated dysfunctions following ablation of senescent cells by AP20187 (Baker et al., 2011). Moreover, another transgenic line, p16-3MR, was generated, where senescent cells can also be eliminated via ganciclovir and further mitigated the post-traumatic osteoarthritis and age-related intervertebral disk degeneration (Jeon et al., 2017; Patil et al., 2019). In addition to transgenic mice, senolytic drugs, including dasatinib and quercetin, ABT263, and SSK1, also showed the therapeutic effects on senescent cells and alleviated the radiation and age-related symptoms and pathology (Cai et al., 2020; Chang et al., 2016; Zhu et al., 2015). A combined treatment of senolytic medicines in transgenic mice also presented the therapeutic effects on obesity-induced metabolic dysfunction and fibrotic pulmonary disease (Palmer et al., 2019; Schafer et al., 2017). Strikingly, two small clinical trials on senolytic treatments with dasatinib and quercetin were completed last year and reported therapeutic effects for patients with diabetic kidney disease (N = 9) and idiopathic pulmonary fibrosis (N = 14) (Hickson et al., 2019; Justice et al., 2019). More recently, other novel senolytic agents such as ABT-737, navitoclax, flavone, fisetin, A1331852, and A1155463 have been developed; ongoing research will determine their effects against various diseases in the future (Yosef et al., 2016; Zhu et al., 2015, 2017).

Physical exercise is widely recognized as a safe, effective, and cost-effective “medicine” for a broad range of age-related diseases. Moreover, a lack of exercise is a major contributing factor to accelerated aging and age-associated chronic conditions, including cancer, obesity, and cardiovascular diseases (Booth et al., 2011). World Health Organization (WHO) thus encourages adults aged 18–64 years to engage in over 150 min of moderate-intensity physical activity each week to reduce the risk of these chronic conditions. Unfortunately, 23% of men and 32% of women worldwide failed to meet this recommendation according to data from WHO, (Guthold et al., 2018). Therefore, a clearer delineation of anti-aging and anti-disease effects and underlying mechanisms of exercise is needed. While the accumulation of senescent cells has been identified as the mechanism of aging and multiple diseases for decades, senolytics targeting senescent cells has just been developed in recent years. In addition, exercise has shown its capacity to lower the marker of senescent cells over the past decade. In the current systematic review of all available literature, we explored the potential senolytic effects of exercise in both human and animal models under healthy or disease states. We aimed to improve the understanding of the cellular senescence-based mechanisms underlying exercise as anti-aging medicine. This may impel people to engage in more exercise and lead to the development of more precise “exercise prescriptions” and “exercise mimetics” for the aging population and patients with various age-related diseases (Li & Laher, 2015).

2 | RESULTS

2.1 | Study characteristics

The systematic search in the database and manually searching the reference list yielded 2182 articles. After the exclusion of duplicates, 1704 articles were screened for the abstract and title. Of these, 60 articles were further screened for full text and finally included 21 articles in this review, which contained eight human studies, twelve animal studies, and one study included both human and animal experiments (Figure 1). The total number of included participants in human studies was 535 (Table 1). Participants, including sedentary or active volunteers, athletes, and patients, aged 18–81 years were recruited. Four out of nine were cohort studies, while three of them were intervention studies.

Overall, most human studies used p16INK4a and T lymphocyte as markers of senescent cells and targeted tissue or cell, respectively. For physical exercise, habitual physical activity (via questionnaire) was determined in most human studies, while resistance training and an acute bout of moderate-high intensity cycling were applied as the exercise intervention. On the other hand, mice (nine studies) and rat (five studies) were utilized as animal models to investigate the senolytic effects of exercise on senescent cells (Table 2). Wild-type and spontaneous or accelerated aging animals were used in most studies. Overall, p16INK4a, p21Cip1, and/or SA-β-Gal were applied as markers of senescent...
cells in various tissues or cells. For chronic exercise training, long-term running, including both wheel/voluntary and treadmill/forced running, was reported in most animal studies, while swimming was also applied as the exercise intervention in three studies.

2.2 Qualitative description of study findings

Of all 21 articles, 16 articles reported senolytic effects of exercise on the markers of senescent cells characterized by declined levels of specific molecular machinery (p16\textsuperscript{INK4a}, p21\textsuperscript{Cip1}, and SA-β-Gal), while five and three articles reported no and opposite effects of exercise on senescent cells, respectively. Interestingly, exercise showed distinct effects on different senescent markers (e.g., p16\textsuperscript{INK4a} and p21\textsuperscript{Cip1}) in one study.

For human studies (Table 1), the level of habitual physical activity is negatively associated with the level of p16\textsuperscript{INK4a} in immune cells of healthy untrained participants aged 18–80 years (Liu et al., 2009; Song et al., 2010; Tsygankov et al., 2009), but not in older coronary bypass patients aged 56–81 years (Pustavoitau et al., 2016). In addition, chronic exercise training reduced the level of p16\textsuperscript{INK4a} or p21\textsuperscript{Cip1} in mononuclear cells or vascular endothelial cells of healthy middle-age athletes (marathon runners or triathletes) and healthy older trained participants compared to sedentary controls (Rossman et al., 2017; Werner et al., 2009). On the other hand, chronic resistance training reduced the level of p16\textsuperscript{INK4a} in aged and overweight/obese women (4 out of 8 participants underwent caloric restriction during training were excluded) (Justice et al., 2018). A single bout of resistance training acutely lowered the level of p16\textsuperscript{INK4a} in the muscle of healthy recreationally active young men (Wu et al., 2019). Collectively, physically active people have lower levels of p16\textsuperscript{INK4a}-positive senescent cells than sedentary people, while resistance training contributed to a lower level of p16\textsuperscript{INK4a}.

For animal studies (Table 2), prolonged voluntary wheel running decreased the level of p16\textsuperscript{INK4a} in various organs/tissues or cells in young, old, or obese mice/rat, including heart, vessel, endothelium, and adipose tissue (Kröller-Schön et al., 2012; Schafer et al., 2016; Werner et al., 2008, 2009), while it remained unchanged in some other organs and tissues, such as kidney and pancreas. Moreover, long-term forced treadmill running showed contradictory effects on the markers of senescent cells, including p16\textsuperscript{INK4a}, p21\textsuperscript{Cip1}, and SA-β-Gal (Bao et al., 2020; Jang et al., 2019; Wong et al., 2019; Yoon et al., 2019; Zhang et al., 2016), although four out of five studies showed senolytic effects of exercise in specific tissues under obesity or aging conditions. Conversely, studies on an acute bout of
| Author (year)          | Participant                                                                 | Exercise                        | PA Levels/Training protocol                                                                 | Organ or cell         | Marker of SCs | Senolytics |
|-----------------------|------------------------------------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------|-----------------------|---------------|------------|
| Liu et al. (2009)     | Healthy participants (18–80 years); n = 170                                  | Habitual PA                     | 0–1900 min/month (n = 170). Comparison between <= 240 and >240 min/month                   | T lymphocyte         | \(p16^{\text{INK}4a} \downarrow\) | Yes        |
| Tsygankov et al. (2009)\(^a\) | Healthy participants (18–80 years); n = 170                              | Habitual PA                     | 0–1900 min/month (n = 170). Comparison between <= 240 and >240 min/month                   | T lymphocyte         | \(p16^{\text{INK}4a} \downarrow\) | Yes        |
| Song et al. (2010)    | Healthy participants (18–80 years); n = 104                                 | Habitual PA                     | >= 0 and <2000 min/month (n = 104)                                                        | T lymphocyte         | \(p16^{\text{INK}4a} \downarrow\) | Yes        |
| Pustavoitau et al. (2016) | Older (56–81 years) patients undergoing coronary artery bypass surgery; n = 47 | Habitual PA                     | >= 0 and <4000 min/month (n = 47). Comparison between <= 240 and >240 min/month           | T lymphocyte         | \(p16^{\text{INK}4a} \rightarrow\) | No         |
| Chronic exercise training                                                                 |                                                                                      |                                                                                             |                                                                                       |                                                                       |             |
| Werner et al. (2009)  | Healthy middle-aged marathon runners or triathletes (51.1 ± 1.6 years) and sedentary control (50.9 ± 1.6 years); n = 46 | Exercise training (running)     | Trained: 80 ± 7.5 km/week for 35 ± 2.7 years; control: < 1 hr per week for 1 year          | Mononuclear cells    | \(p16^{\text{INK}4a} \downarrow\) | Yes        |
| Rossman et al. (2017) | Healthy trained older participants (59 ± 1 years, LTPA: 83 ± 9 MET hr/week) and sedentary control (62 ± 1 years, LTPA: 32 ± 6 MET hr/week); n = 25 | Exercise training               | Trained: >= 45 min/day, 5 days/week for 5 years; control: <30 min/day, 2 days/week for 2 years | Vascular endothelial cell | \(p16^{\text{INK}4a} \downarrow\) & \(p21^{\text{Cip1}} \downarrow\) | Yes        |
| Justice et al. (2018) | Older overweight/obese women (67–77 years); n = 8                         | Resistance training (machine)   | 3 sets of 10 repetitions at 70% 1RM per day, 3 day/week for 5 month                        | Adipose tissue       | \(p16^{\text{INK}4a} \downarrow\) | Yes        |
| Acute exercise bout   |                                                                                      |                                                                                             |                                                                                       |                                                                       |             |
| Yang et al. (2018)    | Healthy recreationally active (> = twice a week for >3 years) college student (22.5 ± 1.7 years); n = 12 | Resistance exercise (squat)     | 6 sets of 12 repetitions at 70% of 1 RM                                                   | Endothelial progenitor cells | \(p16^{\text{INK}4a} \downarrow\) | Yes        |
| Wu et al. (2019)      | Healthy recreationally active men (21 ± 0.2 years); n = 12                   | Bicycle ergometer               | 1 h on bike ergometer at 70% power output                                                 | Muscle                | SA-β-gal→ | No         |

Abbreviations: \(\downarrow\) deceased and \(\rightarrow\) unchanged compared with control (non-exercise); LTPA, leisure time physical activity; MET, metabolic equivalent; PA, physical activity; RM, repetition maximum; SA-β-gal, senescence-associated beta-galactosidase; SCs, senescent cells.

\(^a\)The same cohort as \(\text{Liu et al., 2009}\) used for development of mathematical model that links \(p16^{\text{INK}4a}\) with aging.
| Author (year) | Animal model | Exercise | Exercise protocol | Organ or tissue | Marker of SCs | Senolytics |
|--------------|--------------|----------|-------------------|----------------|--------------|------------|
| **Chronic exercise training** | | | | | | |
| Werner et al. (2008) | Mice (8-week-old); n = 8–12; WT | Wheel running (voluntary) | 5100 ± 800 m/24 h for 21 days | Heart | p16\(^{INK4A}\)↓ | Yes |
| Werner et al. (2009) | Mice (8-week-old); n = 6–8; WT | Wheel running (voluntary) | 4280 ± 670 m/24 h for 21 days | MNC & Vessel | p16\(^{INK4A}\)↓ | Yes |
| Kröller-Schön et al. (2012) | Mice (8-week-old); n = 6; WT | Wheel running (voluntary) | 4336 ± 842 m/24 h for 8 weeks | Endothelium | p16\(^{INK4A}\)↓ | Yes |
| Huang et al. (2013) | Rat (7-week-old); n = 5–6; Accelerated aging | Swimming | 60 min/time, 5 times/week for 12 weeks | Liver | p21\(^{Cip1}\)↓ & SA-\(\beta\)-gal↑ | Opposite & Yes |
| Schafer et al. (2016) | Mice (8-month-old); n = 6–7; Obesity | Wheel running (voluntary) | 3291 ± 1166 m/24 h for 16 weeks | Adipose tissue, liver, aorta, skeletal muscle, pancreas, kidney, heart, | p16\(^{INK4A}\)↓→, p21\(^{Cip1}\)↓→ & SA-\(\beta\)-gal↓→ | Yes & No |
| Zhang et al. (2016) | Rat (20-month-old); n = n.a.; Spontaneous Aging | Treadmill running | 30 min/day at 13 m/min, 5 days/week for 4–8 weeks | Tendons | SA-\(\beta\)-gal↓ | Yes |
| Fan et al. (2017) | Rat (4-month-old); n = 7; Accelerated aging | Swimming | 45 min/day, 5 day/week for 6 weeks | Muscle | SA-\(\beta\)-gal↓ | Yes |
| Yoon et al. (2019) | Mice (9-week-old/19-month-old); n = 5; Spontaneous Aging | Treadmill running | 30 min, twice per day, 5 days/week for 4 weeks | Muscle | p16\(^{INK4A}\)↓→ & p21\(^{Cip1}\)↓→ | Yes & No |
| Wong et al. (2019) | Mice (3–5-month-old/23-24-month-old); n = 5–7; Spontaneous Aging | Treadmill running | 33d at 10 m/min and 5 day at 8 m/min | Epidermal and dermal cell | p16\(^{INK4A}\)↓→ & p21\(^{Cip1}\)↓→ | No |
| Liu et al. (2019) | Rat (8–10-week-old); n = 3; WT | Swimming | 60 min/day for 5 week with sinker weight | Hippocampus cell | p21\(^{Cip1}\)↑ & SA-\(\beta\)-gal↑ | Opposite |
| Jang et al. (2019) | Mice (10-week-old); n = 3; Obesity | Treadmill running | 12-week running, 1 h/day, 5 day/week with increased speed (12–14–15 m/min) | Hippocampus cell | p16\(^{INK4A}\)↓, p21\(^{Cip1}\)↓, SA-\(\beta\)-gal↓ & lipofuscin↓ | Yes |
| Bao et al. (2020) | Mice (19-month-old); n = 3; Spontaneous Aging | Rotatable treadmill running | 15–60 min/day, 5 day/week for 6 weeks with increased time and speed (3.2–4.8 m/min) | Kidney | SA-\(\beta\)-gal↓ | Yes |
| **Acute exercise bout** | | | | | | |
| Saito et al. (2020) | Mice (13-week-old); n = 3–5; WT | Treadmill running | -20° downhill treadmill for 30 min at 17 m/min | Fibro-adipogenic progenitors | p16\(^{INK4A}\)↓→ & p21\(^{Cip1}\)↑ | Opposite & No |

\(^a\)Per group; WT, wild-type; SCs: senescent cells; SA-\(\beta\)-gal: senescence-associated beta-galactosidase; ↑ increased, ↓ deceased and → unchanged compared with control (non-exercise).
downhill running and prolonged swimming reported an increased level of p21Cip1 and SA-β-Gal in fibro/adipogenic progenitors (Saito et al., 2020), and liver and brain (Huang et al., 2013; Liu et al., 2019), respectively, while another swimming study indicated a reduced level of SA-β-Gal in the muscle (Fan et al., 2017). In summary, 10 out of 13 animal studies showed a senolytic effect of exercise, but this effect was influenced by the form and dosage of exercise, type of senescent tissue or cells, and healthy or aging/disease conditions.

2.3 | Meta-analysis

Overall, 16 out of 21 articles were included in the meta-analysis. However, two studies were excluded because of a lack of essential data (Wu et al., 2019; Zhang et al., 2016), one study was excluded because of less than two data with similar outcomes and design (Saito et al., 2020), one study was excluded because the data were taken from cohort reported in another study (Tsygankov et al., 2009), and one study was excluded because of the fact that exercise was not an independent factor (Yang et al., 2018).

For cross-sectional studies in humans, a negative correlation between habitual physical activity and the level of p16INK4a in T lymphocytes ($r = -0.30$, $95\%$ CI = $-0.54, 0.01, I^2 = 84\%$, $p = 0.04$), subgroup by healthy participants ($r = -0.30$, $95\%$ CI = $-0.53, -0.34, I^2 = 0\%$, $p < 0.001$) and patients (one article) were identified (Figure 2a). Moreover, exercise training significantly reduced the level of p16INK4a in humans ($-64\%, 95\%$ CI = $-72\%$, $-55\%$, $I^2 = 0\%$, $p < 0.001$) (Figure 2b).

To identify the source of the high heterogeneity, studies were subgrouped by tissues, including heart, vessel, muscle, fat, skin, brain, liver, pancreas, and kidney. Of these, a senolytic effect of exercise was observed in vessel with a lower heterogeneity ($-56\%$, $95\%$ CI = $-72\%$, $-39\%$, $I^2 = 73\%$, $p < 0.001$), while no significant effect of exercise was found in muscle ($42\%$, $95\%$ CI = $-51\%$, 136\%, $I^2 = 59\%$, $p = 0.37$). For p21Cip1, a significant senolytic effect of exercise was observed in animal studies, though a high heterogeneity was observed ($21\%$, $95\%$ CI = $-32\%$, $-9\%$, $I^2 = 92\%$, $p < 0.001$) (Figure 3). To identify the source of the high heterogeneity, studies were subgrouped by healthy states, including healthy and young, aged, and HFD-induced obesity. Strikingly, no effect of exercise on p21Cip1 was found in a healthy state ($-5\%$, $95\%$ CI = $-14\%$, 4\%), $I^2 = 45\%$,
p = 0.32). In contrast, a significant senolytic effect of exercise on senescent cells was found in obese (~57%, 95% CI = −69%, −46%, I^2 = 62%, p < 0.001) but not aged animals (p = 0.44). SA-β-gal, another marker of senescent cells, was also examined in the animal studies; however, high heterogeneity was observed together with the senolytic effects of exercise, which cannot be reduced by subgroup (~40%, 95% CI = −64%, −16%, I^2 = 99%, p < 0.001) (Figure S2). In summary, senolytic effects of exercise were observed in the vessel (p16^INK4a) and obese animals (p21^Cip1), but not in the muscle (p16^INK4a) or healthy animals (p21^Cip1). In this context, the senolytic effects of exercise remain unclear, especially in various organs or tissues and states. More studies with similar outcomes and designs are needed due to the high level of heterogeneity observed in these studies.

### 2.4 Study quality

The quality scores of each study included in this systematic review are shown in the supplemental materials. For human cross-sectional studies, the score of Newcastle-Ottawa quality assessment ranged from 6 (satisfactory studies) to 8 (good studies) (Table S2). Moreover, AUB KQ1 risk of bias assessment of human intervention studies reported a low risk of random sequence generation (80%), allocation concealment (60%), incomplete outcome data (100%), selective outcome reporting (100%), and other bias (100%), but not blinding of patients and personnel (20%) and blinding of outcome assessment (20%) (Table S3). On the other hand, the median number of CAMARADES checklist score for animal studies was 4, ranging from 3 to 6 (Table S4). Although the quality assessment of animal studies was provided, the risk of bias remained unclear in these studies due to the lack of sufficient details for assessment that has long been identified as the limitation of animal research.

### 2.5 Publication bias

Publication bias was examined for outcomes with over nine data/studies by the asymmetry of funnel plots. No major bias was detected in animal studies on p16^INK4a (Figure S3) or p21^Cip1 (Figure S4), while some asymmetry was observed in the funnel plots, but this was unlikely to be due to publication bias.
Our systematic review and meta-analysis were the first to investigate the senolytic effects of exercise on senescent cells in both human and animal studies. Despite the limited number of studies, the evidence on the senolytic effect of exercise on senescent cells in humans and animals appears convincing. A higher level of habitual physical activity and exercise training decreased the level of p16INK4a in humans. In addition, high heterogeneity was observed in animal studies, probably due to study design and sample heterogeneity rather than a real heterogeneity in results. A further subgroup analysis revealed that exercise reduced the level of p21Cip1 in various organs/tissues under high-fat-diet (HFD)-induced obesity. In contrast, no senolytic effect of exercise on the level of p16INK4a was found in the healthy state. The evidence for other outcomes and conditions is less clear. Thus, more human and animal studies investigating the senolytic effects of exercise on senescent cells are required.

Cellular senescence serves pleiotropic roles in aging, chronic diseases, and cancer (Van Deursen, 2014). In addition to advancing age, unhealthy lifestyles, such as physical inactivity, unhealthy diets, and cigarette smoking, also contribute to the accumulation of senescent cells in the body, especially during aging, and subsequent accelerated aging and chronic diseases (Liu et al., 2009; Song et al., 2010). In this context, the elimination of senescent cells (senolytics) is a potential therapy for aging and chronic diseases, such as obesity, type 2 diabetes, and atherosclerosis (He & Sharpless, 2017). Senolytic medicine has been developed in recent years and some of them are currently undergoing clinical trials, which may require years to be clinically available (Hickson et al., 2019; Justice et al., 2019). Our systematic review and meta-analysis found evidence that exercise has senolytic properties in healthy humans, where a higher level of habitual physical activity or physically active (Liu et al., 2009; Song et al., 2010) and chronic exercise training (Justice et al., 2018; Rossman et al., 2017; Werner et al., 2009) reduced the level of p16INK4a in various tissues/cells, especially the senescent lymphocytes. These findings supported the hypothesis that exercise can be a senolytic medicine, although there was only very limited evidence demonstrating a negative association between habitual exercise/exercise training and the level of senescent markers. Besides, the optimal or the lowest effective dose of exercise remains unclear (Eijssvogels & Thompson, 2015). Therefore, studies on the different forms (e.g., exercise alone or together with other senolytics such as dasatinib and quercetin) and dosages of exercise on senescent cells in various cells/tissues, populations, and health conditions are urgently needed. Moreover, exercise may have potential anti-tumor properties (Pedersen et al., 2016), which raises the question of whether exercise is beneficial for cancer patients in the context of senescent cells as an emerging target of cancer (Lee & Schmitt, 2019). Collectively, physical exercise shows promise as a senolytic medicine against aging and multiple diseases, which calls for more preclinical and clinical studies.

On the other hand, animal studies on the senolytic effects of exercise provided more evidence about its effects on various tissues and organs in detail, which cannot be directly investigated in humans. A recent systematic review and meta-analysis reported that the expression levels of selected markers of senescent cells varied from different tissues during aging (Tuttle et al., 2020). Similarly, we found no significant change of p16INK4a in animal studies with high heterogeneity. However, a significant decline of p16INK4a was detected in the vessels (Kröller-Schön et al., 2012; Schafer et al., 2016; Werner et al., 2009) but not the muscle (Saito et al., 2020; Schafer et al., 2016; Yoon et al., 2019) after subgroup analysis by tissues. In accordance with a previous study that p16INK4a expressed in various organs and tissues, such as the brain, liver, and spleen, but not in the muscle, during aging (Idda et al., 2020), while senescent cells may be the therapeutic target of cardiovascular diseases (Childs et al., 2018). It suggested that senolytic medicine, including exercise, may only be suitable to target the aging and disease states for certain tissues and organs. The comprehensive cellular senescence-based effects of exercise on various organs and tissues remain to be investigated; the results of these studies may contribute to the development of more precise exercise therapeutical strategies in the future.

In addition to p16INK4a, p21Cip1 is another conventional marker of senescent cells. Unlike p16INK4a which is involved in the maintenance of senescent phenotype, p21Cip1 is essential for establishing senescence (Stein et al., 1999). Strikingly, we found exercise showed no effect on the level of p21Cip1 in healthy and young animals (Huang et al., 2013; Schafer et al., 2016; Yoon et al., 2019). Since cellular senescence serve vital roles in health maintenance and cancer suppression (He & Sharpless, 2017), a lack of significant senolytic effects of exercise on p21Cip1 in healthy animals may imply potential safety of using exercise as senolytic medicine, since exercise may only “remove” excess senescent cells induced by pathological stressors. On the contrary, in the HFD-induced obesity, exercise reduced the level of p21Cip1 in obese animals after subgroup analysis by the disease states, including aging and obesity, while more studies are required to understand its effects on senescent cells in aged animals (Jang et al., 2019; Schafer et al., 2016). Cellular senescence is considered to be involved in and serves as the therapeutic target of obesity and metabolic dysfunction (Palmer et al., 2019). However, few disease states have been examined in previous animal studies. Exercise may be a potent and safe senolytic medicine against a specific disease, especially for senescent cells characterized by p21Cip1.

While the senolytic effects of exercise have been identified in the current review, the underlying mechanisms are less well understood. Previous studies have reported that exercise regulated DNA damage (Radak et al., 2002), telomere erosion (Puterman et al., 2010), oxidative stress (Gomez-Cabrera et al., 2008), in various tissues and cells, are the major drivers of cellular senescence. On the other hand, exercise-induced autophagy (He, Bassik, et al., 2012; He et al., 2012) and apoptosis (Phaneuf & Leeuwenburgh, 2001), which have been shown to elicit regulating effects on cellular senescence (Kang et al., 2015; Vicencio et al., 2008), might also be involved in the senolytic effects of exercise. A clearer picture
of mechanisms underlying senolytic effects of exercise may contribute to the discovery and development of “exercise mimetics” or “exercise pills” against senescent cells (Li & Laher, 2015) and encourage people to engage in more exercise to tackle physical inactivity as one of the leading health problems in the world (Blair, 2009; Kohl et al., 2012) and to develop exercise as an effective treatment for specific diseases.

The present systematic review and meta-analysis provide an up-to-date summary of the senolytic effects of exercise on senescent cells based on both human and animal studies. However, there are some limitations that should be mentioned. The major limitation of this study is the limited number of studies included in this review, although the effects of exercise on senescent cells have been studied for over a decade. In addition, the relatively high heterogeneity was observed among studies due to the complexity of research on cellular senescence, such as the types of cells/tissues and markers of senescent cells. Besides, the search strategy was limited to the articles written in English, which may lead to a language bias and a potential loss of studies met other items of inclusion criteria.

The findings of this systematic review and meta-analysis provided some evidence that exercise may be a senolytic medicine for p16INK4a-positive senescent cells in humans and for p21Cip1-positive senescent cells in obese but not healthy animals. Future studies should examine the optimal form and dosage of exercise, targeted cells/tissues, different disease states, and the underlying cellular mechanisms in humans and animals. A greater understanding of the senolytic effects of exercise can lead to significant clinical and public health impact.

4 | METHODS

4.1 | Search strategy and data sources

A systematic search of the literature was conducted on May 20, 2020, in three databases: MEDLINE (OVID), EMBASE (OVID), and Web of Science using the Medical Subject Headings (MeSH) terms “exercise” and “cellular senescence” without date limit (a full list of search items used in systemic search is listed in Table S1). Articles were imported into Mendeley reference management software (Elsevier, USA), and the duplicates were identified and removed. Titles and abstracts of all identified records were independently screened by two reviewers to exclude irrelevant articles. The specific inclusion criteria were as follows: (a) original study; (b) peer-reviewed article; (c) articles written in English; (d) full text available; (e) animal or human studies; (f) “senescence” or “senescent” means “cellular senescence”; (g) “exercise” means physical exercise. Subsequently, full text of the remaining articles was retrieved and reviewed by the same independent reviewers with the following additional inclusion criteria: (a) markers of senescent cells (p16INK4a, p21Cip1, SA-β-Gal, and lipofuscin) were reported; (b) exercise as a sole intervention/contributing factor; (c) for immunosenescence, articles were included if cellular senescence in immune cells was reported.

4.2 | Study quality assessment

The quality of included studies was assessed independently by two reviewers using (a) Newcastle-Ottawa quality assessment scale for cohort studies, (b) AUB KQ1 risk of bias assessment, and (c) collaborative approach to meta-analysis and review of animal data from experimental studies (CAMARADES) checklist (Anon n.d.) for (a) habitual physical activity studies in humans, (b) exercise training studies in humans, and (c) animal studies, respectively. The information of each article was then summarized after independent assessment and the disagreement was resolved by discussion.

4.3 | Extraction of data

Data were extracted independently by the same reviewers. Any disagreement was resolved by discussion or a third reviewer. The following study characteristics were extracted onto a pre-designed data collection form: author, publication year, participant or animal characteristics (e.g., age, health and exercise status, type of animal), sample size, types of exercise, physical activity level/training protocol, types of organ, tissue or cell, markers of senescent cells and their responses to exercise, senolytic effect (yes, no, or opposite). For analysis purposes, means, standard deviation, and sample size were collected for each study. If the data were reported in graphical form, means and standard deviation were extracted using WebPlotDigitizer (Rohatgi, 2011). Studies were excluded if the mean, standard deviation or sample size was not available or equal to 0. If the sample size was provided as a range, we utilized the smallest value. To examine the effects of exercise in different markers of senescent cells, these data were extracted separately. Moreover, to determine whether the senolytic effects of exercise were specific to certain disease state, data from healthy/young samples and disease/old samples were extracted separately as well. Therefore, the total number of data points included in the meta-analysis was greater than the number of articles.

4.4 | Data analysis

For all meta-analyses, review manager (RevMan) version 5.3 (Copenhagen: The Nordic Cochrane Center) was used where at least two studies of similar study design had reported the same outcomes (i.e., markers of senescent cells). For each study, the mean differences based on the changes from control to exercise group, or from baseline to post-exercise, were calculated and pooled using the random-effects model. Correlation coefficient (r) was used to calculate the effect size after transforming to Fisher’s z in meta-analysis Fisher’s z was then transformed back to r for presentation. Heterogeneity was determined using Cochrane’s chi-square test and Higgins’s I² test while publication bias was explored using funnel plots. To assess the specific senolytic effects of exercise on senescent cells in animal studies, we pre-defined the following subgroups.
for analyses: markers of senescent cells, healthy and young/disease or old, and organ/tissue. Sensitivity analysis was performed by removing the lowest quality study among included studies from the analysis.

ACKNOWLEDGMENT

XKC is supported by a postgraduate studentship by the Graduate School, The University of Hong Kong. The study is partially supported by a research grant provided by the Department of Medicine.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

XKC, RCC, ACM, and JSK contributed to the conception and design of this study. XKC, ZNY, and KMH contributed to literature searching and eligible study screening. XKC and ZNY contributed to the data extraction and analysis. XKC, ZNY, KMH, GTW, RCC, ACM, and JSK contributed to the manuscript preparation and reviewed the final version.

DATA AVAILABILITY STATEMENT

We believe this systematic review is exempt under the rule of data availability.

ORCID

Kazi Md. Mahmudul Hasan https://orcid.org/0000-0001-5724-8347
Raymond Chuen-Chung Chang https://orcid.org/0000-0001-8538-7993

REFERENCES

Anon Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies. Retrieved from http://www.dcn.ed.ac.uk/camarades/default.htm [Accessed July 21, 2020].

Baker, D. J., Wijshake, T., Tchkonia, T., LeBrasseur, N. K., Childs, B. G., Van De Sluis, B., Kirkland, J. L., & van Deursen, J. M. (2011). Clearance of p16 Ink4a-positive senescent cells delays ageing-associated disorders. Nature, 479, 232–236.

Bao, C., Yang, Z., Li, Q., Cai, Q., Li, H., & Shu, B. (2020). Aerobic endurance exercise ameliorates renal vascular sclerosis in aged mice by regulating PI3K/AKT/mTOR signaling pathway. DNA and Cell Biology, 39, 310–320.

Ben- Porath, I., & Weinberg, R. A. (2005). The signals and pathways activating cellular senescence. International Journal of Biochemistry & Cell Biology, 37, 961–976.

Blair, S. N. (2009). Physical inactivity: the biggest public health problem of the 21st century. British Journal of Sports Medicine, 43, 1–2.

Booth, F. W., Roberts, C. K., & Laye, M. J. (2011). Lack of exercise is a major cause of chronic diseases. Comprehensive Physiological, 2, 1143–1211.

Cai, Y., Zhou, H., Zhu, Y., Sun, Q., Ji, Y., Xue, A., Wang, Y., Chen, W., Yu, X., & Wang, L. (2020). Elimination of senescent cells by β-galactosidase-targeted prodrug attenuates inflammation and restores physical function in aged mice. Cell Research, 30, 574–589.

Chang, J., Wang, Y., Shao, L., Laberge, R.-M., Demaria, M., Campisi, J., Janakiraman, K., Sharpless, N. E., Ding, S., & Feng, W. (2016). Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nature Medicine, 22, 78–83.

Childs, B. G., Durik, M., Baker, D. J., & van Deursen, J. M. (2015). Cellular senescence in aging and age-related disease: From mechanisms to therapy. Nature Medicine, 21, 1424–1435.

Childs, B. G., Li, H., & Van Deursen, J. M. (2018). Senescent cells: A therapeutic target for cardiovascular disease. Journal of Clinical Investigation, 128, 1217–1228.

Collado, M., Blasco, M. A., & Serrano, M. (2007). Cellular senescence in cancer and aging. Cell, 130, 223–233.

Eijsvogels, T. M. H., & Thompson, P. D. (2015). Exercise is medicine: at any dose? JAMA, 314, 1915–1916.

Fan, J., Yang, X., Li, J., Shu, Z., Dai, J., Liu, X., Li, B., Jia, S., Kou, X., Yang, Y., & Chen, N. (2017). Spermidine coupled with exercise rescues skeletal muscle atrophy from D-gal-induced aging rats through enhanced autophagy and reduced apoptosis via AMPK-FOXO3a signal pathway. Oncotarget, 8, 17475–17490.

Gomez-Cabrera, M.-C., Domenech, E., & Viña, J. (2008). Moderate exercise is required for muscle glucose homeostasis. Nature, 481, 511–515.

He, C., Sumpter Rhea, J., & Levine, B. (2012). Exercise induces autophagy in peripheral tissues and in the brain. Autophagy, 8, 1548–1551.

Hickson, L. T. J., Langhi Prata, L. G. P., Bobart, S. A., Evans, T. K., Gior gadze, N., Hashimi, S. K., Herrmann, S. M., Jensen, M. D., Jia, Q., Jordan, K. L., Kellogg, T. A., Kholis, S., Koerber, D. M., Lagnado, A. B., Lawson, D. K., LeBrasseur, N. K., Lerman, L. O., McDonald, K. M., McKenzie, T. J., ... Kirkland, J. L. (2019). Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. EBioMedicine, 47, 446–456.

Huang, C. C., Chiang, W. D., Huang, C. W., Huang, C. Y., Hsu, M. C., & Lin, W. T. (2013). Hepatoprotective effects of swimming exercise against D-galactose-induced senescence rat model. Evidence-Based Complementary and Alternative Medicine, 2013, (no p.). Retrieved from http://easyaccess.lib.cuhk.edu.hk/login?url=http://ovidsp.ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed14&AN=36932192275431.

Idda, M. L., McClusky, W. G., Lodde, V., Abdelmohsen, K., Rossi, M., & Gorospe, M. (2020). Survey of senescent cell markers with age in human tissues. Aging (Albany NY), 12, 4052–4066.

Jang, Y., Kwon, I., Cosio-Lima, L., Wirth, C., Vinci, D. M., & Lee, Y. (2019). Endurance exercise prevents metabolic distress-induced senescence in the hippocampus. Medicine and Science in Sports and Exercise, 51, 2012–2024.

Jeon, O. H., Kim, C., Laberge, R.-M., Demaria, M., Rathod, S., Vassermot, A. P., Chung, J. W., Kim, D. H., Poon, Y., & David, N. (2017). Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nature Medicine, 23, 775–781.

Justice, J. N., Gregory, H., Tchkonia, T., Lebrasseur, N. K., Kirkland, J. L., Kritchevsky, S. B., & Nicklas, B. J. (2018). Cellular senescence biomarker p16 INK4a + cell burden in thigh adipose is associated with poor physical function in older women. The Journal of Gerontology: Series A, 73, 939–945.
Justice, J. N., Nambiar, A. M., Tchkonia, T., LeBrasseur, N. K., Pascual, R., Hashmi, S. K., Prata, L., Masternak, M. M., Kritchevsky, S. B., Musi, N., & Kirkland, J. L. (2019). Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. *Ebiomedicine*, 40, 554–563.

Kang, C., Xu, Q., Martin, T. D., Li, M. Z., Demaria, M., Aron, L., Lu, T., Yankner, B. A., Campisi, J., & Elledge, S. J. (2015). The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. *Science*, 349, 6255. https://doi.org/10.1126/science.aaa5612

Kirkland, J. L., Tchkonia, T., Zhu, Y., Niedernhofer, L. J., & Robbins, P. D. (2017). The clinical potential of senolytic drugs. *Journal of the American Geriatrics Society*, 65, 2297–2301.

Koh, J., Rebbapragada, K., Vlahos, D., Serrano, M., & Johnson, T. (2019). Targeting senescent cells ameliorates age-related macular degeneration. *Aging Cell*, 18, e12950.

Liu, B., Li, H., Xu, L., Vlahos, D., Serrano, M., & Johnson, T. (2019). Targeting senescent cells ameliorates age-related macular degeneration. *Aging Cell*, 18, e12950.

Patil, P., Dong, Q., Wang, D., Chang, J., Wiley, C., Demaria, M., Lee, J., Kang, J., Niedernhofer, L. J., & Robbins, P. D. (2019). Systemic clearance of p16INK4a-positive senescent cells mitigates age-associated intervertebral disc degeneration. *Aging Cell*, 18, e12927.

Pedersen, L., Idrorn, M., Olofsson, G. H., Lauenborg, B., Nookaew, I., Hansen, R. H., Johannesen, H. H., Becker, J. C., Pedersen, K. S., Dethlefsen, C., Nielsen, J., Gehl, J., Pedersen, B. K., thron, P., & Hofman, P. (2016). Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. *Cell Metabolism*, 23, 554–562. Retrieved from http://www.sciencedirect.com/science/article/pii/S1550413116300031

Phaneuf, S., & Leeuwenburgh, C. (2001). Apoptosis and exercise. *Medicine and Science in Sports and Exercise*, 33, 393–396.

Pustavoitau, A., Barodka, V., Sharpless, N. E., Torrice, C., Nyhan, D., Berkowitz, D. E., Shah, A. S., Roche, K. J. B., & Walston, J. D. (2016). Role of senescence marker p16INK4a measured in peripheral blood T-lymphocytes in predicting length of hospital stay after coronary artery bypass surgery in older adults. *Experimental Gerontology*, 74, 29–36.

Puterman, E., Lin, J., Blackburn, E., O’Donovan, A., Adler, N., & Epel, E. (2010). The power of exercise: buffering the effect of chronic stress on telomere length. *PLOS One*, 5, e10837.

RADÁK, Z., Naito, H., Kaneko, T., Tahara, S., Nakamoto, H., Takahashi, R., Cardozo-Peleaze, F., & Goto, S. (2002). Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. *Pflügers Archiv European Journal of Physiology*, 445, 273–278.

Rodier, F., & Campisi, J. (2011). Four faces of cellular senescence Four faces of senescence. *Journal of Cell Biology*, 192, 547–556.

Rohatgi, A. (2011). WebPlotDigitizer. Retrieved from http://arohatgi.info. WebPlotDigitizer/app

Rossman, M. J., Kaplan, R. E., Hill, S. D., McNamara, M. N., Santos-Parker, J. R., Pierce, G. L., Seals, D. R., & Donato, A. J. (2017). Endothelial cell senescence with aging in healthy humans: Prevention by habitual exercise and relation to vascular endothelial function. *American Journal of Physiology-Heart and Circulatory Physiology*, 313, H890–H895.

Saito, Y., Chikenji, T. S., Matsumura, T., Nakano, M., & Fujimiya, M. (2020). Exercise enhances skeletal muscle regeneration by promoting senescence in fibro-adipogenic progenitors. *Nature Communications*, 11. Retrieved from http://easyaccess.lib.cuhk.edu.hk/login?url=http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=e&emexa&AN=51579889. https://doi.org/10.1038/s41467-020-14734-x

Schafer, M. J., White, T. A., Evans, G., Tonne, J. M., Verzosa, G. C., Stout, M. B., Mazula, D. L., Palmer, A. K., Baker, D. J., Jensen, M. D., Torbenson, M. S., Miller, J. D., Ikeda, Y., Tchkonia, T., van Deursen, J. M., Kirkland, J. L., & LeBrasseur, N. K. (2016). Exercise prevents diet-induced cellular senescence in adipose tissue. *Diabetes*, 65, 1606–1615.

Schafer, M. J., White, T. A., Iijima, K., Haak, A. J., Ligresti, G., Atkinson, E. J., Oberg, A. L., Birch, J., Salmonowicz, H., & Zhu, Y. (2017). Cellular senescence mediates fibrotic pulmonary disease. *Nature Communications*, 8, 14532.

Song, Z. F., von Figura, G., Liu, Y., Kraus, J. M., Torrice, C., Dillon, P., Rudolph-Wataabe, M., Ju, Z. Y., Kestler, H. A., Sanoff, H., & Rudolph, K. L. (2010). Lifestyle impacts on the aging-associated expression of biomarkers of DNA damage and telomere dysfunction in human blood. *Aging Cell*, 9, 607–615.

Stein, G. H., Drulinger, L. F., Soulard, A., & Dulić, V. (1999). Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. *Molecular and Cellular Biology*, 19, 2109–2117.

Tchkonia, T., Morbeck, D. E., Von Zglinicki, T., Van Deursen, J., Lustgarten, J., Scoble, H., Khosla, S., Jensen, M. D., & Kirkland, J. L. (2010). Fat tissue, aging, and cellular senescence. *Aging Cell*, 9, 667–684.

Tsygankov, D., Liu, Y., Sanoff, H. K., Sharpless, N. E., & Elston, T. C. (2009). A quantitative model for age-dependent expression of the p16INK4a tumor suppressor. *Proceedings of the National Academy of Sciences*, 106, 16562–16567.

Tuttle, C. S. L., Waaijer, M. E. C., Slee-Valentijn, M. S., Stijnen, T., Dethlefsen, C., Nielsen, J., Gehl, J., Pedersen, B. K., thron, P., & Hofman, P. (2016). Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. *Cell Metabolism*, 23, 554–562. Retrieved from http://www.sciencedirect.com/science/article/pii/S1550413116300031

Vicenzi, J. M., Galluzzi, L., Tajedidine, N., Ortiz, C., Crillo, A., Tasdemir, E., Morselli, E., Ben Younes, A., Mairui, M. C., Lavandero, S., & Kroemer, G. (2008). Senescence, apoptosis or autophagy? *Gerontology*, 54, 92–99.

Werner, C., Forster, T., Widmann, T., Poss, J., Roggia, C., Hanhoum, M., Schragh, J., Buchner, N., Meyer, T., Kindermann, W., Haendeler, J., Bohm, M., & Laufs, U. (2009). Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. *Circulation*, 120, 2438–2447. Retrieved from http://easyaccess.lib.cuhk.edu.hk/login?url=http://ovidsp.ovid.com/ovidw
Zhang, J., Yuan, T., & Wang, J. H. C. (2016). Moderate treadmill running exercise prior to tendon injury enhances wound healing in aging rats. Oncotarget, 7, 8498–8512.

Zhu, Y., Doornebal, E. J., Pirtskhalava, T., Giorgadze, N., Wentworth, M., Fuhrmann-Strossnigg, H., Niedernhofer, L. J., Robbins, P. D., Tchkonia, T., & Kirkland, J. L. (2017). New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. Aging (Albany NY), 9, 955–963. https://doi.org/10.18632/aging.101202.

Zhu, Y. I., Tchkonia, T., Pirtskhalava, T., Gower, A. C., Ding, H., Giorgadze, N., Palmer, A. K., Ikono, Y., Hubbard, G. B., Lenburg, M., O’Hara, S. P., LaRusso, N. F., Miller, J. D., Roos, C. M., Verzosa, G. C., LeBrasseur, N. K., Wren, J. D., Farr, J. N., Khosla, S., ... Kirkland, J. L. (2015). The Achilles’ heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell, 14, 644–658.

How to cite this article: Chen XK, Yi ZN, Wong GC, et al. Is exercise a senolytic medicine? A systematic review. Aging Cell. 2021;20:e13294. https://doi.org/10.1111/acel.13294

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.