Low-dose quercetin positively regulates mouse healthspan

Dear Editor,

Aging is the leading risk factor for many chronic diseases, accounting for almost 60% of all deaths worldwide. How to achieve healthy aging, alleviate aging-related diseases, and extend healthspan has become a main topic of biomedical research (He et al., 2019). Geroprotective compounds, such as metformin and rapamycin, have been shown to improve both healthspan and lifespan in mice (Martin-Montalvo et al., 2013; Bitto et al., 2016), whereas nicotinamide partially improves healthspan in mice (Mitchell et al., 2018). In addition, senolytics, compounds that eliminate senescent cells, have been proven to improve physical function and increase lifespan in mice (Xu et al., 2018). Although none have proven to be clinically reliable in delaying aging or treating frailty in humans, these compounds have already provoked enthusiasm for identifying a potential “elixir”. Therefore, the exploration of more geroprotective compounds, especially natural active compounds, holds great potential for the development of geriatric medicines.

Quercetin (Que) is a natural bioflavonoid found in fruits and vegetables such as apples and onions. Que (50 mg/kg) in combination with dasatinib (5 mg/kg) (abbreviated as D + Q) has been shown to effectively eliminate senescent cells via induction of apoptosis, thus alleviating senescence-related phenotypes and improving physical function and lifespan in mice (Zhu et al., 2015; Xu et al., 2018). In addition, Que (10 mg/kg) in combination with dasatinib (5 mg/kg) has been reported to reduce hepatic steatosis (Ogrodnik et al., 2017). In each of these in vivo studies, however, Que was used at high doses ranging from 10 to 50 mg/kg body weight, which raises concerns about dose-dependent side effects such as headaches and limb tingling (Shoskes et al., 1999). As a selective tyrosine kinase receptor inhibitor, dasatinib is associated with warnings and precautions including pulmonary arterial hypertension and low blood cell counts. Therefore, high-dose Que and extra side effects of dasatinib would hamper potential clinical applications of Que in geriatric medicines. Through natural products screening using Werner syndrome (WS) human mesenchymal stem cells (hMSCs), we recently identified Que as a geroprotective agent that counteracts accelerated and natural aging of hMSCs at a concentration of as low as 100 nmol/L, which is 100 times lower than the concentration of Que (10 μmol/L) previously used in combination with dasatinib as senolytic drugs to eliminate senescent cells in human umbilical vein cells (HUVECs) (Zhu et al., 2015; Geng et al., 2018).

To explore the geroprotective effect of low-dose Que monotherapy in rodents, we evaluated the in vivo effect of long-term low-dose Que administration under physiological-aging condition. Que was given to 14-month-old C57BL/6J male mice by weekly oral gavage at a concentration of 0.125 mg/kg body weight, which is 80–400 times lower than that of the previously tested D + Q (10–50 mg/kg body weight) regimens (Fig. 1A), with vehicle (10% PEG400 in PBS)-treated mice as controls (Zhu et al., 2015; Xu et al., 2018). After eight months of treatment, Que-treated mice showed decreased hair loss with normal food intake, body weight, blood glucose and bone mineral density (Figs. 1B and S1A–D). Compared to vehicle-treated mice, mice subjected to Que treatment showed markedly improved exercise endurance in the RotaRod and treadmill tests, but normal grip strength by grip strength meter assay (Figs. 1C, 1D, and S1E–G). Accordingly, the cardiac function of these mice was examined by Doppler tissue imaging. Although ejection fraction (EF) and fractional shortening (FS) were unaffected, a higher frequency of the mitral ratio of peak early to late diastolic filling velocity (E/A) within the normal range was observed in Que-treated mice than in the age-matched controls (Figs. 1E and S1H). However, the lifespan was not prolonged by low-dose Que treatment observed up to the age of 31 months (Fig. S1I). Taken together, these data indicate that long-term low-dose Que administration alone sufficiently improves multiple aspects of healthspan, but not lifespan, in mice.

To investigate how Que improved healthspan in mice, we collected 11 different kinds of tissues from 10-week young male mice (Y-Ctrl) and vehicle (O-Veh)- and low-dose Que-treated 22-month old male mice (O-Que). No significant difference was observed in organ weights between O-Veh and O-Que (Fig. S2A). Given that exercise endurance and diastolic function were improved by Que, we particularly examined the changes in skeletal muscles (SKM), white adipose tissues (WAT), brown adipose tissues (BAT) and hearts. Upon Que treatment, the arrangement of muscle fibers became more regular and compact with less fibrosis...
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LETTER

A

Quercetin

B

Relative area of hair loss (fold)

C

Hanging time (min)

D

Frequency of electric shock (times)

E

Velocity mm/s

Y-Ctrl O-Veh O-Que

E/A < 1.3 E/A = 1.3 ~2.5 E/A > 2.5 E/A = 1.3 ~2.5 E/A > 2.5

Y-Ctrl 0 12 0 100%
O-Veh 3 4 5 33.33%
O-Que 4 8 0 66.67%

F

Relative area of fibrosis (fold)

G

Adipocyte area (μm²)

SA-β-Gal positive region (fold)

Log10(normalized read count +1)

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Accordingly, mobilization of RTEs is likely to be a key con- 

hMSCs (Geng et al., 2018). Constitutive heterochromatin senescence in part through the restoration of heterochro-

mRNA-seq of SKM, WAT, and BAT. Scale bar, 100 μm (n = 4). Data are shown as the mean ± SEM. *P < 0.05. (D) Frequency of electric shock on the treadmill over 30 min (n = 12). Data are shown as the mean ± SEM. *P < 0.05. (E) The ratio of peak velocity of early to late filling of mitral inflow (E/A) (n = 12). The table shows the number of mice in 3 kinds of E/A ranges, and Que treatment increased the ratio of normal E/A mice. *P < 0.05. (F) Masson’s trichrome staining in SKM showed moderate perivascular and interstitial fibrosis (blue areas) (n = 4). Data are shown as the mean ± SEM. *P < 0.05. Scale bar, 100 μm. SA-β-Gal staining analysis of SKM, WAT and BAT. Scale bar, 100 μm (n = 4). Data are shown as the mean ± SEM. **P < 0.01, *P < 0.05. Haematoxylin and eosin staining of WAT. Scale bar, 100 μm (n = 4). Data are shown as the mean ± SEM. *P < 0.05. (G) Global gene expression profiling in SKM, WAT and BAT (n = 3). Y-Ctrl represents 10-week-old young male mice, and O-Veh and O-Que represent vehicle (10% PEG400 in PBS)- or low-
dose Que-treated old male mice.

and senescence (Figs. 1F and S2B). In WAT, the increases in adipocyte size and senescence-associated β-galactosi-
dase (SA-β-Gal)-positive area during aging were both alle-
viated upon Que treatment (Fig. 1F). In BAT, although adipocyte size was unaffected, there was a decreasing trend of the SA-β-Gal-positive area upon Que treatment (Figs. 1F and S2B). By comparison, we did not observe any significant differences in mouse hearts by histological analysis and SA-β-Gal staining (Fig. S2B). Therefore, these data suggest that long-term low-dose Que administration may delay aging of SKM, WAT, and BAT in mice.

To further explore the molecular mechanisms of the ben-
eficial effects of Que, we performed whole-transcriptome RNA sequencing (RNA-seq) of SKM, WAT, and BAT from Y-Ctrl, O-Veh, and O-Que mice. Global gene expression profiling revealed that most protein coding genes were unaffected after long-term low-dose Que administration (Fig. 1G). Accordingly, we inferred that low-dose Que might exert its senostatic effect by regulating the expression of non-protein-coding RNAs.

We previously observed that Que alleviates hMSC senescence in part through the restoration of heterochromatin architecture in prematurely and physiologically aging hMSCs (Geng et al., 2018). Constitutive heterochromatins are predominantly comprised of repetitive elements (REs), including retrotransposable elements (RTEs). The expression of RTEs is repressed via epigenetic regulation under normal conditions but is elevated during physiological aging, eliciting active transposition (De Cecco et al., 2013). Accordingly, mobilization of RTEs is likely to be a key contributor to tissue aging and cell degeneration (De Cecco et al., 2013). To investigate whether low-dose Que treatment antagonized the activation of RTEs, we examined the expression levels of various RTEs, including long terminal repeats such as LTR10C, LTR2C, LTR35A, and LTR3B, and non-long terminal repeats including long interspersed nuclear elements 1 (L1, also known as LINE-1) and short interspersed nuclear elements (SINEs) such as Alu in WS hMSCs after continuous Que treatment at the concentration of 100 nmol/L. Que treatment silenced the transcription of various RTEs in WS hMSCs, consistent with the rejuvenated cellular phenotypes (Fig. 2A). To test whether Que treatment may also repress activation of RTEs in a mouse in vivo model, we compared the transcriptional levels of RTEs such as L1, SINE B1, LTR41, LTR42 and MLV5 in multiple tissues of Y-Ctrl, O-Veh, and O-Que mice. Consistently, most RTEs were transcriptionally upregulated in the SKM and BAT of old mice compared to those of young mice and were repressed by Que treatment (Fig. 2B). Similar to the tendency in the BAT, RTEs in WAT from Que-treated mice were also slightly decreased (Fig. 2B). In line with enhanced L1 transcripts, there was an increased expression level of L1 open reading frame 1 protein (ORF1p) in SKM and BAT of aged mice, which could be reversed by long-term low-dose Que administration (Fig. 2C–E). These data indicate that Que represses RTE activation in senescent hMSCs and multiple aged mouse tissues.

In senescent cells, the activation of RTEs (such as L1) leads to genome instability and accumulation of cytosolic DNA that further binds to cytosolic sensor cGAS and acti-
vates TBK1 and IRF3, which subsequently promote senes-
cence-associated secretory phenotype (SASP) (Takahashi et al., 2018; De Cecco et al., 2019). In addition, NF-κB/RelA in cGAS-STING-mediated NF-κB pathway acts with IRF3 and other transcription factors to induce the expression of inflammatory cytokines such as IL-6, the most prominent SASP cytokine (Chen et al., 2016). Notably, both p-TBK1 and p-IRF3 were increased in old mouse tissues compared to the young ones and were repressed upon Que treatment (Fig. 2E), indicating the effect of Que on inhibiting cGAS-
STING pathway (Kato et al., 2017). Similarly, RelA (p65) was upregulated in aged mouse tissues and repressed upon Que treatment (Fig. 2E–G). Consistently, the inflammatory cyto-
kine IL-6 was increased in old mice compared to young mice and Que antagonized the increase of IL-6 in both WS-
hMSCs and old mouse SKM and BAT (Fig. 2A and 2B). Thus, our data suggest that Que may block SASP through the axis of heterochromatin-RTEs (L1)-innate immune response pathway (Fig. 2H). In this study, we reported for the first time a geroprotective effect of low-dose quercetin alone that improved the healthspan of aged C57BL/6J male mice. Que-treated mice showed less hair loss, greater athletic endurance, enhanced diastolic function, and less muscle fibrosis, as well as allevi-
vated cellular senescence in multiple tissues. Interestingly, these changes appear to be rarely associated with trans-
criptional alterations of protein-coding genes but are linked
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to heterochromatin stabilization and RTE silencing. Que treatment prevented L1 from hyperactivation, thereby inhibiting SASP. In contrast to the reported senolytic effect of high-dose D+Q (Xu et al., 2018), where Que exerts geroprotective activity (De Cecco et al., 2019). Que has been proven as a potential inhibitor of reverse transcriptase activity against RTEs in hMSCs and rodents (Ono et al., 2019). Our data provide important evidence supporting the role of low-dose Que in safeguarding genomic stability (i.e. inhibition of retrotransposition), which at least in part contributes to its geroprotective activity in rodents.

FOOTNOTES

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REFERENCES

Bitto A, Ito TK, Pineda VV, LeTexier NJ, Huang HZ, Sutlief E, Tung H, Vizzini N, Chen B, Smith K et al (2016) Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. eLife 5:e16351
De Cecco M, Criscione SW, Peterson AL, Neretti N, Sedivy JM, Kreiling JA (2013) Transposable elements become active and mobile in the genomes of aging mammalian somatic tissues. Aging 5:867–883
De Cecco M, Ito T, Petrashen AP, Elias AE, Skvir NR, Criscione SW, Caligiana A, Broccoli G, Adney EM, Boeke JD et al (2019) L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature 566:73–78
Geng L, Liu Z, Zhang W, Li W, Wu Z, Wang W, Ren R, Su Y, Wang P, Sun L et al (2018) Chemical screen identifies a geroprotective role of quercetin in premature aging. Protein Cell 10(6):417–435
He X, Song M, Qu J, Guo Y, Cao H, Sun R, Liu G-H, Shen Y, Major Program Expert G (2019) Basic and translational aging research in China: present and future. Protein Cell. https://doi.org/10.1007/s13238-019-0617-0
Martin-Montalvo A, Mercenko EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, Gomes AP, Ward TM, Minor RK, Bloin MJ et al (2013) Metformin improves healthspan and lifespan in mice. Nat Commun 4:2192
Mitchell SJ, Bernier M, Aon MA, Cortassa S, Kim EY, Fang EF, Palacios HH, Ali A, Navas-Enamorado I, Di Francesco A et al (2018) Nicotinamide improves aspects of healthspan, but not lifespan, in mice. Cell Metab 27:667–676.e664
Ogrodnik M, Miwa S, Tchkonia T, Tiniakos D, Wilson CL, Lahat A, Day CP, Burt A, Palmer A, Anstee QM et al (2017) Cellular senescence drives age-dependent hepatic steatosis. Nat Commun 8:15691–15691
Ono K, Nakane H, Fukushima M, Cherrmann J-C, Barré-Sinoussi F (1990) Differential inhibitory effects of various flavonoids on the activities of reverse transcriptase and cellular DNA and RNA polymerases. Eur J Biochem 190:469–476
Shoskes DA, Zeitlin SI, Shahed A, Rafier J (1999) Quercetin in men with category III chronic prostatitis: a preliminary prospective, double-blind, placebo-controlled trial. Urology 54:960–963
Simon M, Van Meter M, Ablaeva J, Ke Z, Gonzalez RS, Taguchi T, De Cecco M, Leonova KL, Kogan V, Helfand SL et al (2019) LINE1 derepression in aged wild-type and SIRT6-deficient mice drives inflammation. Cell Metab 29:871–885.e875
Takahashi A, Loo TM, Okada R, Kamachi F, Watanabe Y, Wakita M, Watanabe S, Kawamoto S, Miyata K, Barber GN et al (2018) Downregulation of cytoplasmic DNases is implicated in cytoplasmic DNA accumulation and SASP in senescent cells. Nat Commun 9:1249
Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, Inman CL, Ogrodnik MB, Hachfeld CM, Fraser DG et al (2018) Senolytics improve physical function and increase lifespan in old age. Nat Med 24:1246–1256
Zhang W, Li J, Suzuki K, Qu J, Wang P, Zhou J, Liu X, Ren R, Xu X, Ocampo A et al (2015) A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging. Science (New York, NY) 348:1160–1163
Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, Palmer AK, Ikeno Y, Hubbard GB, Lenburg M et al (2015) The Achilles’ heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 14:644–658

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