Muscular Swedish mutant APP-to-Brain axis in the development of Alzheimer’s disease

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INTRODUCTION
Alzheimer’s disease (AD) is the most common form of dementia. Notably, patients with AD often suffer from severe sarcopenia. However, their direct link and relationship remain poorly understood. Here, we generated a mouse line, TgAPPsweHSA, by crossing LoxP-STOP-LoxP-APPswe with HSA-Cre mice, which express APPswe (Swedish mutant APP) selectively in skeletal muscles. Examining phenotypes in TgAPPsweHSA mice showed not only sarcopenia-like deficit, but also AD-relevant hippocampal inflammation, impairments in adult hippocampal neurogenesis and blood brain barrier (BBB), and depression-like behaviors. Further studies suggest that APPswe expression in skeletal muscles induces senescence and expressions of senescence-associated secretory phenotypes (SASPs), which include inflammatory cytokines and chemokines; but decreases growth factors, such as PDGF-BB and BDNF. These changes likely contribute to the systemic and hippocampal inflammation, deficits in neurogenesis and BBB, and depression-like behaviors, revealing a link of sarcopenia with AD, and uncovering an axis of muscular APPswe to brain in AD development.

Cell Death and Disease (2022)13:952 ; https://doi.org/10.1038/s41419-022-05378-4

Although APPswe is detected in small fractions of EOAD patients, its functions in Aβ production in the brain and in promoting AD pathogenesis have been well studied in multiple animal models (e.g., Tg2576 and 5XFAD, both well-characterized AD animal models that express APPswe under the control of prion and Thy1 promoter, respectively). Second, much AD research has been focused on the impact of Aβ on the brain, even though App or APPswe is known to be expressed not only in the brain, but also in periphery tissues [15, 16], including skeletal muscles [17]. While investigating phenotypes in APPswe-based animal models have provided valuable insights into Aβ pathology in the brain and impairments in mouse cognitive functions, the functions of APPswe in periphery tissues, such as muscles, remain poorly understood. Third, APP’s physiological function in muscles has been emerged. In addition to its age-dependent expression in muscles and NMJs (neuro-muscular junction) [17], mice with APP knocking out show dysfunctional NMJs with aberrant localization of presynaptic proteins, reduced synaptic vesicles, and abnormal postsynaptic AChR clusters [18]. Fourth, altered expression or increased cleavage of APP appears to be involved in multiple types of human muscle degenerative diseases (see Supplemental Table 1). For examples, muscle fibers of patients with inclusion-body myositis (IBM) have intra-fiber “plaque-like” Aβ accumulation [19], which are believed to promote myofiber degeneration, atrophy, and death [20]. Aβ accumulation in strophic muscles fibers is a key factor in GNE myopathy [21]. Aβ accumulation is also detected in muscles of patients with ALS (amyotrophic lateral...
sarcopenia) and ALS mouse models [22]. In AD patients, Aβ levels are elevated not only in the brain, but also in muscles (e.g., temporalis) [23]. Finally, examinations of muscle structures in Tg2576, the well-characterized AD animal model that expresses APPswe ubiquitously, have revealed early-onset sarcopenia-like deficits, months before any brain-pathologic defect that can be detected [24, 25]. Taken together, these observations argue against the view for sarcopenia-like deficits as a consequence of neurodegeneration, or a random coincidence, implicate dysfunctional APP or Aβ as a potential common denominator for AD and muscle degenerative diseases, and raise additional question—could problems in muscles contribute to AD pathology in the brain?

Here, we addressed this question by use of a newly generated APPswe-based animal model, TgAPPswe:HSA-Cre mice, which express APPswe specifically in muscles. Investigating phenotypes in TgAPPswe:HSA mice showed not only earlier onset sarcopenia-like muscle deficits, but also age-dependent depression-like behaviors and brain pathology (largely in the hippocampus), such as increased glial activation, impaired BBB, and elevated pro-inflammatory cytokines. Further studies demonstrate increased senescence and SASPs in APPswe expressing muscles or C2C12 cells; and inhibition of senescence by its inhibitors diminishes or abolishes nearly all the phenotypes in TgAPPswe:HSA mice. These results thus demonstrate a contribution of muscular APPswe to the development of both AD and sarcopenia, revealing a link between sarcopenia and AD, and uncovering a muscle-to-brain crosstalk for AD development.

MATERIALS AND METHODS
The LSL-APPswe mice were generated using the pCAGL2 plasmid as described previously [26]. TgAPPswe:HSA mice were generated by crossing LSL-APPswe with HSA-Cre mice (purchased from Jackson laboratory, which is donated by Dr. IMR Colony, stock #006149) [27]. This study was conducted in accordance with the Institutional Animal Care and Use guidelines of the Institutional Animal Care and Use Committee at Case Western Reserve University approved protocols (IACUC, 2017–0121 and 2017–0115).

The other material and methods including Animals, Reagents, Behavioral tests, Stereological cell counting, Histologic staining (H&E, NADH-TR (transferease), SDH (succinate dehydrogenase), COX (cytochrome c oxidase), PAS (Periodic Acid Schiff), and Gomori-trichrome), Immunofluorescence staining and image analysis, Western blotting, EdU injection and labeling, L-Series label-multiplex antibody arrays, SA-β-gal staining, Elisa assays, RNA isolation, and RT-qPCR were described in supplemental information.

RESULTS
TgAPPswe:HSA, a mouse model that selectively expressing APPswe in skeletal muscles
To investigate possible skeletal muscular APPswe’s effect in AD and sarcopenia development, we generated TgAPPswe:HSA or LSL-APPswe:HSA-Cre mice by crossing LSL(Loxp-Stop-Loxp)-hAPP (human APPswe) with HSA-Cre (human skeletal α-actin promoter driven Cre) mice (Fig. S1A). The APPswe expression in TgAPPswe:HSA mice is thus under the control of both the CAG promoter (a chicken β-actin promoter with a CMV enhancer to express its mRNAs) and the HSA-Cre dependent removal of LSL in LSL-hAPP mice (Fig. S1A) [26]. The specific expression of the HSA-Cre activity in the skeletal muscles was verified in HSA-Cre: Ai9 mice (where the tdTomato expression depends on Cre activity) (Fig. S1B), in line with literature reports [27]. We further examined hAPPswe’s expression in TgAPPswe:HSA mice. RT-qPCR analysis using specific primers for human APP detected hAPPs transcripts only in skeletal muscles, but un-detectable in the brain-hippocampus or cortex, nor other tissues/organs (such as heart, lung, liver, and kidney) of TgAPPswe:HSA mice (Fig. S1C). Among different muscles, the hAPPs transcripts were abundantly expressed in tibialis anterior (TA) (a distal fast twitch type), Quadriceps (a proximal fast twitch type), and soleus (a slow twitch type) in TgAPPswe:HSA mice (Fig. S1C). Western blot analysis also showed selectively expression of hAPPs protein in TA muscles, but not in the cortex nor hippocampus in TgAPPswe:HSA mice (Fig. S1D, E), verifying the RT-qPCR results.

Decreased muscle fiber size and increased central nuclei in TgAPPswe:HSA mice in muscle fiber type- and age-dependent manners
Given the abundant expression of APPswe in skeletal muscles, we wondered whether such muscular APPswe expression could induce sarcopenia-like deficits, such as decreased muscle fiber size and increased muscle fiber degeneration [28, 29]. H&E and histologic staining analysis of TA muscle fibers showed normal or comparable morphology in 3-MO TgAPPswe:HSA mice to those of control mice (Fig. 1A, B). However, at 6-MO, the mutant TA muscles exhibit sarcopenia-like deficits, showing smaller muscle fiber area with increased fibers containing central nuclei (Fig. 1A, B), a feature of muscle fiber degeneration [30]. We then characterized the phenotypes in other type of muscles, including quadriceps and soleus. While the mutant quadriceps, a proximal fast twitch type of muscles, showed similar age-dependent deficits to those of mutant TA muscles (Fig. 1C, D), the mutant soleus, a slow twitch type of muscles, exhibited an earlier onset degenerative phenotype, exhibiting a reduction in their fiber size, and an increase in fibers with central nuclei distribution at age of 3-MO (Fig. 1E, F), which were un-detectable in the mutant TA nor quadriceps at this age (Fig. 1).

These results thus suggest muscle fiber type- and age-dependent sarcopenia-like deficits in TgAPPswe:HSA mice. This view was further tested by additional histologic staining analyses, including Gomori-trichrome, PAS (Periodic Acid Schiff), NADH-Transference, and COX (cytochrome c oxidase), and SDH (succinate dehydrogenase), in both 3- and 6-MO TA and soleus muscles. Indeed, at both ages of 3- and 6-MO, the mutant soleus muscles showed increases in fibers with Gomori-trichrome positive staining, decreased COX+, but increased COX-SDH+ fibers, and elevated cytoplasmic PAS− fibers (Fig. S2), suggesting mitochondrial myopathy, fibrosis, and glycogen overload in the mutant soleus. The mutant TA muscles also showed decreased COX− and increased COX-SDH+ fibers at both 3- and 6-MO, elevated cytoplasmic PAS− fibers at 6-MO, but little changes by Gomori-trichrome and NADH-TR staining (Fig. S2), suggesting a relatively weaker myopathy in TA than those in soleus muscles from the mutant mice. Together, these results provide additional support for earlier onset of myopathies in the mutant muscles, which resemble the features of sarcopenia-like myopathy.

Age-dependent increases in hippocampal reactive astrocytes and microglia in TgAPPswe:HSA mice
We then asked whether TgAPPswe:HSA mice exhibit any brain pathology similar to those of APPswe-based AD animal models (e.g., Tg2576) [31–33]. It is known that APPswe-based AD animal models (e.g., Tg2576) exhibit not only increased Aβ40 and Aβ42 levels in the brain, but also elevated glial activation, inflammation, and reduced neuronal synapses [31–33]. We thus first measured both Aβ40 and Aβ42 levels in muscles, serum samples, and brain tissues in TgAPPswe:HSA mice (at 6-MO), compared with those of same aged LSL-APPswe mice (a negative control) and Tg2576 (a positive control) mice. ELISA analyses of Aβ40 or Aβ42 levels showed little difference in the brain, cortex, or serum samples, but a slight increase in the TA muscles, of TgAPPswe:HSA mice (6-MO), as compared with those of the negative control mice (Fig. S1F, G). In contrast, Tg2576 mice showed marked increases of Aβ40 and Aβ42 levels in their brain tissues and serum samples, and a comparable level of Aβ40 in TA muscles to that of TgAPPswe:HSA mice (Fig. S1F, G).

We second examined neuronal distribution patterns and densities in the hippocampus and cortex of TgAPPswe:HSA mice (at 6-MO) through a co-immunostaining analysis using antibodies.
against NeuN (a marker for all neurons) and Ctip2 (a marker for neurons in the Layers V-VI cortex and CA1-2 and DG hippocampus). Little to no changes in the NeuN$^+$ and Ctip2$^+$ neuron distribution patterns and densities were detected in TgAPP$^+$HSA brains, as compared with those of controls (Fig. S3).

Third, we assessed the morphologies and densities of glial cells, including Olig2$^+$ oligodendrocytes, S100$^+$β$^+$ ependymal cells, GFAP$^+$ astrocytes, and IBA1$^+$ microglial cells, in the brain sections of control (LSL-APP$^+$swe) and TgAPP$^+$swe HSA mice. Again, little to no changes in the Olig2$^+$ oligodendrocytes or S100$^+$β$^+$ ependymal cells were detected in the brain of TgAPP$^+$swe HSA mice (Fig. S4). However, both GFAP$^+$ astrocytes and IBA1$^+$ microglial cells were increased in the 6-MO TgAPP$^+$swe HSA brain, particularly in the hippocampus at both dorsal and ventral regions (Fig. 2A–H), suggesting an activation of these glial cells. In line with this view, the increased GFAP and IBA1 protein levels were also detected in 6-MO TgAPP$^+$swe HSA hippocampus using Western blot analysis (Fig. 2I, J). Notice that GFAP$^+$ astrocytes and IBA1$^+$ microglial cells were slightly increased in the cortex layer I-III of 6-MO TgAPP$^+$swe HSA (Fig. S5A–C), but the protein levels remained unchanged in the 6-MO TgAPP$^+$swe HSA cortex by Western blot analysis (Fig. S5D, E). This suggests that the hippocampus appeared to be more vulnerable than the cortex in the mutant mice. Negligible changes of these glial cells at 3-MO were observed in the mutant brain (Fig. S6), indicating an age-dependency of these phenotypes.

Increases in expression of inflammatory cytokines, but decreases in growth factors and BBB integrity in the hippocampus of 6-MO TgAPP$^+$swe HSA mice

Considering the tight association of glial cell activation with brain inflammation [13, 34], we examined expressions of inflammatory cytokines (e.g., Il1β, Il6, Il10, and Tnfa), chemokines (e.g., Ccl3, 5, 12, 17), and growth factors (e.g., Pdgfb, Bdnf, Tgfb1 and Csf2) in the hippocampus of both control and TgAPP$^+$swe HSA mice (at 3/6-MO) using RT-qPCR analysis (Fig. S7A, B). Among 12 genes examined in 3-MO TgAPP$^+$swe HSA mice, only Bdnf was decreased, as compared with that of control mice (Fig. S7A). However, among the 61 genes examined in 6-MO TgAPP$^+$swe HSA mice, 17 were increased and 8 were decreased in the mutant hippocampus (Fig. S7B). Interestingly, hippocampus of Tg2576 mice (6-MO) showed a similar inflammation phenotype to that of TgAPP$^+$swe HSA mice, displaying increased expression of Il6, Il15, Cxcl10, Lif, and Vegfd, but decreased expression of Pdgfb and Bdnf (Fig. S7C, D).

Notice that PDGF-BB is a key growth factor for the development of pericytes, a blood vessel associated cells that regulate BBB integrity [35, 36]. The reduction of Pdgfb in the mutant hippocampus led to a speculation for a deficiency in PDGFRβ$^+$ pericytes. Indeed, co-immunostaining analysis showed decreased PDGFRβ$^+$ pericytes, but little to no changes in the CD31 marked endothelial cells, in the mutant hippocampus (at 6-MO) (Fig. 3A–C). We then examined BBB leakage by tail vein injections of...
Dextran (3 kDa) into the control and mutant mice (at 6-MO) (Fig. 3D). The dextran signals outside of CD31<sup>+</sup> blood vessels were detected in the mutant hippocampus, but not in the control (Fig. 3E). Interestingly, the dextran signals were largely in the mutant Hilus region (Fig. 3D), indicating a selective BBB leakage in this region. Moreover, more IBA1<sup>+</sup> microglial cells were associated with CD31<sup>+</sup> blood vessels in the mutant Hilus than those of the control mice (Fig. 3F, G), supporting the notion that blood vessel/BBB are damaged in this region.

Age-dependent impairment in adult hippocampal neurogenesis in TgAPP<sub>swe</sub>HSA mice

Given the reports that BDNF is a critical growth factor for adult hippocampal/DG (dentate gyrus) neurogenesis [37–39], and considering the reduction of Bdnf in not only AD animal models [40], but also TgAPP<sub>swe</sub>HSA hippocampus (Fig. S7), we examined DG neurogenesis in the mutant mice. EdU was injected into the mice (at ages of 1-, 3- and 6-MO, respectively, ~12 h before sacrifice) to label proliferative neural stem cells (NSCs). Hippocampal sections were co-immunostained with EdU and antibody against DCX (doublecortin), a marker for newborn neurons derived from NSCs, as shown in Fig. S8A. Remarkably, TgAPP<sub>swe</sub>HSA mice at ages of 3-MO and 6-MO, but not 1-MO, displayed significant reductions in EdU<sup>+</sup> and DCX<sup>+</sup> cell densities at both dorsal and ventral DG (Fig. S8), demonstrating an early onset deficit in the NSC proliferation and thus DG neurogenesis in TgAPP<sub>swe</sub>HSA mice, exhibiting another similar deficit as AD animal models [40].
We then asked whether TgAPPswe function, and working memory, respectively [41, 42] (Figs. 4A, MO, both males and females) to Morris water maze (MWM) and Y-maze, for the assessment of mouse spatial learning and memory function, and working memory, respectively [41, 42] (Figs. 4A–C and S9). No obvious difference in MWM or Y-maze task performance was observed between the mutant and control mice (Figs. 4A–C and S9), suggesting little cognitive decline in TgAPPswe mice.

Age-dependent depression-like behaviors in TgAPPsweswe mice

We then asked whether TgAPPsweswe mice show any behavior changes similar to those of AD animal models (e.g., Tg2576), such as age-dependent impairment in cognitive function [32, 33]. We first subjected TgAPPsweswe and control (LSL-APPsweswe) mice (at age of 6-MO, both males and females) to Morris water maze (MWM) and Y-maze, for the assessment of mouse spatial learning and memory function, and working memory, respectively [41, 42] (Figs. 4A–C and S9). No obvious difference in MWM or Y-maze task performance was observed between the mutant and control mice (Figs. 4A–C and S9), suggesting little cognitive decline in TgAPPsweswe mice.

Anxiety- and depression-like behaviors are often associated with increased glial activation and inflammatory cytokines, and decreased DG neurogenesis. We thus subjected mice (at ages of 3- and 6-MO) to behavior tests including open field test (OFT) to evaluate TgAPPsweswe mice’ locomotor activity, anxiety, and willingness to explore environments [43]; elevated plus maze test (EPM) and light/dark transition test (LDT) to assess mouse anxiety-related behavior [43–45]; sucrose preference test (SPT), force swimming test (FST), and tail suspension test (TST) to examine mouse depression [45, 46]. As shown in Fig. 4D–G, TgAPPsweswe mice developed age-dependent depression-like behaviors. At age of 3-MO, no obvious difference in all the behavior tests was detected between mutant and control mice (Fig. S10). However, at 6-MO, the mutant male mice exhibited increased immobility times in both FST and TST, and decreased sucrose preference (Fig. 4G), without obvious changes in the performance of OPT, EPM, and LDT (Fig. 4D–F), suggesting depression-like behavior, but little deficits in exploratory and locomotor activities and anxiety in 6-MO TgAPPsweswe mice. These depression-like behaviors were detectable not only in male, but also in female mutant mice (Fig. S9).

Chronic systemic inflammation in TgAPPsweswe mice

To understand how expression of APPsweswe in skeletal muscles in TgAPPsweswe mice affects their brain inflammation and function, we tested a speculation that the inflammation and depression-like behavior phenotypes of TgAPPsweswe mice may be induced by secreted proteins from APPsweswe muscles. We first screened for altered serum plasma proteins in TgAPPsweswe mice (at 6-MO) using multiplex antibody-based arrays (Fig. 5A). Among the 90 proteins on the array, only 3 were lower, but 42 were higher in the mutant serum samples than those of control mice (Fig. 5A, B).

We second examined 3-MO mutant mice and compared the changes in their serum samples with those of 6-MO mutant mice. Using the same multiplex antibody-based arrays, 24 proteins were increased, and 14 were decreased in 3-MO mutant mice (Fig. 5C). Comparing the changes between 6-MO and 3-MO mutant mice, 17 proteins were increased, and 2 proteins were decreased at both 3- and 6-MO mutant serum samples (Fig. 5D, E). Interestingly, among these 17 increased proteins, 12 of them exhibited more dramatic increases in 6-MO than those of 3-MO mutant mice (Fig. 5E). These results suggest age-dependent changes in serum proteins in TgAPPsweswe mice.

Third, we addressed whether TgAPPsweswe mice (at 6-MO) exhibit similar changes in their serum samples to those of 6-MO Tg2576 mice. Remarkably, among 42 upregulated proteins in the serum of TgAPPsweswe mice, 29 were also elevated in Tg2576 mice (Fig. 5F).

Finally, among the altered serum proteins in both TgAPPsweswe and Tg2576 mice, two pathways were noted: one is the increased pro-inflammatory cytokine (e.g., IL6 and IL1β) and chemokine pathway, and the other is the decreased growth factor (e.g., PDGF-BB) pathway. We thus used ELISA to further verified changes in IL6, IL1β, and PDGF-BB in serum samples of both mouse lines. Indeed,
both IL6 and IL1β cytokines were increased in TgAPPsweHSA and Tg2576 serum samples (Fig. 5G); and PDGF-BB was significant lower in the serum samples of 6-MO TgAPPsweHSA and Tg2576 mice compared to those of control mice (Fig. 5H), but not in 3-MO TgAPPsweHSA mice (Fig. 5H). Together, these results reveal a similar, but not identical, profile change in TgAPPsweHSA serum samples to those of Tg2576 mice, providing evidence for a chronic systemic inflammation in both TgAPPsweHSA and Tg2576.

**Increased senescence and SASPs in TgAPPsweHSA muscles**

Given APPswe's specific expression in skeletal muscles in TgAPPsweHSA mice, we speculate that the APPswe muscles might be the key...
source for the increased systemic inflammation. We thus analyzed transcripts of the altered genes in the 6-MO control and TgAPPsweHSA TA muscles. Among the 61 genes examined by RT-qPCR analyses, 37 up- and 6 down-regulated transcripts were detected in TgAPPsweHSA TA muscles (Fig. S11A). Interestingly, 49 factors were tested in both serum and TA muscle samples. 30 factors in serum and 27 factors in muscle were upregulated in mutant mice, and 19 (~63%) of them were increased in the mutant TA muscles (Fig. S11B). Comparing the altered transcripts between mutant muscles and hippocampus showed 12 upregulated transcripts (e.g., Il6, Lif, Csf1) and 3 downregulated transcripts (e.g., Pdgfb, Bdnf, and Il4) in both tissues (Fig. S11C, D). Whereas these results support the view for a systemic inflammation in the muscle-blood-hippocampus axis, further correlation plots showed a significant correlation of the significant changes of these transcripts between mutant TA muscles vs hippocampus (Fig. S11E), but not mutant TA muscles vs serum (Fig. S11F), nor mutant serum vs hippocampus (Fig. S11G).

Notice that the increased cytokines and chemokines in the mutant mouse muscle/serum/hippocampal samples exhibit features of senescence-associated secretory phenotype (SASP) [47, 48]. We thus asked if APPswe+ muscles exhibit increased expressions of senescence marker proteins, p16ink4a and p53. RT-qPCR analysis showed that both p16ink4a and p53 were increased in all three muscles (TA, quadriceps, and soleus), but not in other tissues/ organs of TgAPPsweHSA mice at age of 3-MO (Fig. 6A). However, at age of 6-MO, in addition to these muscles, the mutant hippocampus, but not other tissues/organs, showed elevated expression of p16ink4a and p53 (Fig. 6B). These results suggest age-dependent and muscle and hippocampus selective cellular senescence. The increased muscle and hippocampal senescence in the mutant mice were further verified by Western blot analysis (Fig. 6C, D) and co-immunostaining analysis using antibodies against p53 (Fig. 6E–G and Fig. S12). Notice that the increased P53+ immunosignals were selectively detected in the mutant hippocampal hilus region, but not cortex of TgAPPsweHSA mice (Fig. 6E–G and Fig. S12), in line with the view for the hilus region to be a more vulnerable region in response to the stress induced by the muscle APPswe. In addition, we verified the cellular senescence phenotype induced by expressing APPswe in C2C12 cells (Fig. 6H–K). C2C12 cells (a muscle cell line) expressing APPswe-GFP, but not APPswe-GFP nor YFP showed increased SA-β-Gal (another marker for cellular senescence) (Fig. 6H, I), p16ink4a and p53 (Fig. 6J, K), indicating the specificity of the detrimental effect induced by the overexpression of APPswe.

Attenuations of muscle and hippocampal pathologies, systemic inflammation, and behavior phenotypes in TgAPPsweHSA mice treated with senolytic drugs

We next asked whether inhibition of senescence in TgAPPsweHSA mice could diminish the brain and behavior phenotypes. A combination of Dasatinib (D) and Quercetin (Q) was used to inhibit senescence, due to their well examined senolytic effectiveness in animal studies [49, 50]. TgAPPsweHSA mice at 3-MO were administered D + Q to then be subjected to phenotypic examinations at 6-MO (Fig. S13A). We first verified D + Q's effect on muscle senescence in TgAPPsweHSA mice. As shown in Fig. S13B, C, muscles from TgAPPsweHSA mice treated with D + Q showed reduced expressions of senescence markers, p53 and p16ink4a, confirming D + Q's inhibitory effect. We also examined D + Q's effect on various types of cells in TA muscles of TgAPPsweHSA mice. In addition to muscle fibers, muscle tissue/organ contain nerve terminals (e.g., NMJ-neuromuscular junction),
adipocytes, macrophages, and blood vessels [51, 52]. Oil Red O-stained adipocytes in the mutant TA muscles were comparable to that of controls (data not shown); however, CD11b+ macrophages were significantly increased in the mutant muscles (Fig. S13D, E). Interestingly, D + Q treatments abolished the increase of CD11b+ macrophages (Fig. S13D, E), increased muscle fiber size, and reduced central nuclei+ degenerative muscle fibers (Fig. S13F, G). Furthermore, we examined D + Q’s effect on SASP-like factors in muscles of TgAPPswetmHSA mice, which were largely reduced by the D + Q treatments (Fig. 7A). Together, these results suggest that muscle SnCs and the activated macrophages may contribute to the expression of these SASP factors as well as the sarcopenia-like muscle deficit.

We then determined whether the hippocampal phenotypes in TgAPPswetmHSA mice were diminished by D + Q treatments. Remarkably, the phenotypes including glial activation, elevated vessel associated microglia, increased SASP-like factors (e.g., Lif, Il5, Il15, Ccl9, and Ccxl9), and decrease of growth factors (e.g., Pdgfd and Bdnf) and PDGFRβ+ pericytes, in TgAPPswetmHSA hippocampus were all diminished by D + Q treatments (Fig. 7A). Moreover, the impaired hippocampal DG neurogenesis in TgAPPswetmHSA mice was restored by D + Q (Fig. S14G, H).

We further measured serum SASP-like cytokines and chemokines, and growth factors (e.g., PDGFB) in TgAPPswetmHSA mice with and without D + Q treatments. Notice that many cytokines (IL1β, IL3, IL4, IL7, IL23, TNFA, and TREM-1) and chemokines (CCL2, 3, 4, 4, 11, 12, 17, and CXCL10, 11, 13) were increased, and PDGF-BB was decreased in the serum samples of TgAPPswetmHSA mice treated with Vehicle (Fig. S15 and Fig. 7C). Those changes were all normalized by D + Q treatments (Fig. S15 and Fig. 7C), suggesting that the systemic inflammation in TgAPPswetmHSA mice is in large due to the APPswetm-induced senescence and SASPs.

Finally, we compared behavior responses in TgAPPswetmHSA mice treated with or without D + Q. The depression-like behaviors by TST, FST, and SPT were also diminished in the mutant mice by D + Q treatments (Fig. 7D). Taken together, these results suggest that APPswetm-induced senescence and SASPs are likely to prompt systemic and hippocampal inflammation, glial activation, and BBB leakage largely in the Hilus-hippocampus, which may underlie the depression-like behavioral phenotypes in 6-MO TgAPPswetmHSA mice (Fig. 7E and Table 1).

**DISCUSSION**

Here, we use the TgAPPswetmHSA mouse model that selectively expresses APPswetm in skeletal muscles and provide evidence for muscular APPswetm’s contributions to sarcopenia-like deficit, as well as AD-relevant brain pathology. We further investigated the mechanisms underlying muscular APPswetm’s detrimental functions. Our results, summarized in Table 1, lead to a working hypothesis depicted in Fig. 7E, in which, muscular APPswetm promotes sarcopenia-like deficit, AD-relevant hippocampal pathology, and...
depression-like behavior likely due to the increased senescence and SASPs, which appear to be a driver for the systemic and hippocampal inflammation, and thus expedites hippocampus pathology and depression-like behaviors in TgAPPsweHSA mice. These observations thus reveal a link of sarcopenia with AD, and uncover a muscular APPswe to brain axis in AD development.

How does APPswe in muscle cells induce brain/hippocampal pathology? We propose that APPswe-induced muscle senescence and SASPs may underlie its effects on the brain/hippocampus via systemic inflammation (Fig. 7E). Many SASP-like proteins were induced not only in APPswe+ muscles, but also in serum samples and hippocampus of TgAPPsweHSA mice (Figs. 5, S7, and S11). Regarding
the systemic inflammation in TgAPP<sub>swe</sub>HSA mice, our results suggest that APP<sub>swe</sub>-induced senescence and SASPs in muscles appear to be a key contributor to this event. Many (19 over 30, ~63%) upregulated SASP-like factors are detected in both muscles and serum samples of TgAPP<sub>swe</sub>HSA mice (Fig. S11B). Many (29 over 42, ~66%) increased serum proteins in TgAPP<sub>swe</sub>HSA mice were also detectable in the Tg2576 mouse serum samples (Fig. S5F). Inhibition of senescence by its inhibitors (D+Q) abolished most of the increased inflammatory cytokines in the serum samples of TgAPP<sub>swe</sub>HSA mice (Fig. S15 and Fig. S7C). However, further correlation analyses showed a significant association of SASP-like factors’ expression levels in TgAPP<sub>swe</sub>HSA TA muscles with their hippocampus (Fig. S11E), but not with their serum samples (Fig. S11F). We speculate that such un-correlation may be due to the different factors more complex in muscle cells, and thus make the elevated SASP-like factors complex in TgAPP<sub>swe</sub>HSA serum samples than those in TgAPP<sub>swe</sub>HSA TA muscles. We also speculate that the dramatic effect on the systemic inflammation by APP<sub>swe</sub> expression in muscles may be due to the abundant muscle tissues in the body, which account for 30–40% of a person’s body weight; and the consideration of muscles as a critical endocrine organ [53].

In addition, the hypothesis is also in line with our results that various types of muscles from TgAPP<sub>swe</sub>HSA mice showed increased senescence cells as early as 3-MO (Fig. 6F and Fig. S12). Expression of APP<sub>swe</sub> but not APP<sub>wt</sub> in C2C12 cells also increased senescence cells (Fig. 6H, I). These defects occurred at the same age or earlier than the brain (largely hippocampus) phenotypes in TgAPP<sub>swe</sub>HSA mice (Figs. 1, 2 and Fig. 5S). Moreover, inhibition of senescence in TgAPP<sub>swe</sub>HSA mice attenuated nearly all the hippocampal and behavior phenotypes (Fig. 7D and Fig. S14).

In addition to the increased SASP-like proteins (largely cytokines and chemokines), there are reductions in a few of growth factors, such as Pdgfb and Bdnf (Fig. S11A). The reduced Pdgfb and Bdnf were detected only in muscles, but also in the hippocampus, of TgAPP<sub>swe</sub>HSA mice (Fig. S7B). The decreased PDGF-BB was also observed in the serum samples of TgAPP<sub>swe</sub>HSA mice (Fig. S5H). Interestingly, the inhibition of senescence restored PDGFB-BB levels in TgAPP<sub>swe</sub>HSA mice (Fig. S15E, F). These results suggest that APP<sub>swe</sub> induced muscle senescence not only increases SASP-like proteins but also reduces these growth factors, which may also contribute to hippocampal pathology, especially BBB deficit, in the mutant mice. In light of above observations, we speculate that the mutant muscle may derive and increase pro-inflammatory cytokines (e.g., IL6) and decreased growth factors (e.g., BDNF and PDGF-BB) may play important roles in inducing cellular senescence, glial cell activation, and BBB deficit in the mutant hippocampus-in particular the Hillus region. Such a brain-region selective effect may be due to the abundantly expression of their receptors in hippocampal neurons, pericytes, and/or glial cells, which make hippocampus-Hillus region more vulnerable to the stress induced by these upregulated cytokines and/or downregulated growth factors. The receptors. In line with this view are reports that BDNF receptor-TrkB [54], PDGFb receptor [55], and cytokine receptor-check IL6’s receptor [56] are abundantly expressed in hippocampus. In addition, the hippocampal DG area has more neural stem cells, another feature making it more vulnerable to the stress-induced senescence. This view is also in line with multiple literature reports that link cellular senescence with muscle and brain aging, and various degenerative diseases, including AD [57–62]. Several papers also demonstrate the use of senolytic drugs to attenuate the disease progression in several AD animal models [63, 64]. However, how APP<sub>swe</sub> in muscles induces senescence and SASPs remains unclear. We hope to address this question in future studies.

In summary, the results presented in this paper suggest a multi-cell and multi-organ model for AD development in which skeletal muscle cells may serve as a nidus of the disease. This study may reveal an important muscle-to-brain axis, where APP<sub>swe</sub>-induced muscle cell-senescence accelerates brain cell aging and neurodegeneration, opening new avenues to explore interactions between muscles and brain cells during AD development and progression.

DATA AVAILABILITY
Data will be made available on reasonable request.

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