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APN deficiency accelerates brain aging via mitochondrial associated neuroinflammation

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Abstract

A wide spectrum of changes occur in the brain with age, from molecular to morphological aspects, and inflammation accompanied by mitochondria dysfunctions is one of the significant processes and factors associated with age. Adiponectin (APN), an adipokine important in glucose and lipid metabolism, is involved in the aging; however, their roles in brain aging have not been explored adequately. Here, we aimed to mechanistically illuminate the causal relation of brain aging with APN deficiency via multiple biochemical and pharmacology approaches while using APN KO mice, primary microglia, and BV2 cells. Initially, we found that declined APN levels in aged subjects correlated with dysregulated cytokine levels in human individuals. Interestingly, APN KO mice exhibited accelerated aging accompanied by learning and memory deficits, anxiety-like behaviors, neuroinflammation, and immunosenescence. APN deficient mice displayed aggravated mitochondrial dysfunction and HDAC1 upregulation. Accordingly, adiponectin receptor agonist AdipoRon could alleviate the mitochondrial deficits and aging markers induced by rotenone or antimycin A in BV2 cells. Further results indicated that HDAC1 antagonism by Cpd 60 reduced the mitochondrial dysfunction and improved aging-related inflammation, as validated in D-galactose treated APN KO mice. Altogether, these findings indicate that APN is a critical regulator of aging by preventing neuroinflammation associated with mitochondrial impairment of the brain via HDAC1 signaling.

Introduction

Aging is the unavoidable time-dependent decline of organ functionality leading to diseases and death. As the average age expectancy increases, its consequences and influences become apparent and enhanced [11]. Therefore, humans seek ways to prolong life and promote healthy aging. The modern longevity concerns are molecular signalings which may stretch a clue to manipulate the aging process and mechanism [1, 23].

Among various aging-related hypotheses, mitochondrial impairment is considered a key hallmark of aging [21, 28]. As highly active cells, neurons have high energy demands to perform their activities and are thus particularly sensitive to proper mitochondrial function. Mitochondrial dysfunctions result in imbalanced ROS levels associated with oxidative stress, altered ATP production, and inflammatory disorders involved in the aging process, indicating that mitochondrial defects are the early key initiators during aging [15, 19]. Recent studies reveal that epigenetic alterations as histone deacetylases (HDACs) have been linked to mitochondrial hemostasis and longevity, whereas histone deacetylases promote the expression of genes involved in the mitochondrial stress response, immunity, and metabolism. Thus, HDACs 'role in mitochondrial stress response is beneficial regarding longevity.

Besides energy metabolism, mitochondrial is a well-known primary mediator of inflammation, and impaired mitochondria can modulate innate immunity via redox-signaling or direct inflammasome activation [20]. The adult brain maintains the balance between pro-and anti-inflammatory cytokines. This balance shifts to pro-inflammatory with increased age, making the aging brain more vulnerable to stress

and diseases [31]. Increased cytokines, including IL-6, activated microglial cells, and inflammatory signalings like NF-kB expression have been reported in the aged brain, indicating the role of dysregulated mitochondria as the driver of the inflammatory process. [37].

Metabolically aging is also characterized by changes in body composition, such as the decline in growth hormones and insulin resistance[3]. Significant impairments of adipose lipogenesis, adipokines, and cytokine levels are associated with aging and contribute to the onset of aging-related diseases[1, 3]. Adipose tissues deficiency and lipodystrophy are associated with dysregulated adipokines, which provides the base for age-associated adverse metabolic consequences, including insulin resistance, hyperglycemia, dyslipidemia, indicating that the adipose endocrine system is critically important for maintaining whole-body physiology[33]. Thus, metabolic regulators, including adipokines, are critical players in aging consequences and pathologies. Adiponectin (APN) is the most abundant adipocytokine primarily secreted by adipose tissues and regulates various physiological processes via its receptors (AdipoR1 and AdipoR2) [9]. It has antioxidative and anti-inflammatory effects in multiple cells under different pathological conditions, apart from regulating glucose and lipid metabolism. Further, it has been evidenced that APN is paradoxically enhanced in older individuals. Similarly, high circulating APN levels are reported in several long-lived mouse mutants[10], indicating it's linked with aging and associated pathologies. However, the detailed mechanisms underlying aging-related neuroinflammation and adiponectin dysregulation have yet to be determined[32].

The present study aimed to illuminate the interplay between mitochondrial impairment and APN during aging. The potential roles of APN and its association with mitochondrial impairment in aging processes were explored. Contrary to APN levels, increased HDAC1 expression was found in the aged subjects, accompanied by neuroinflammation associated-mitochondrial and cognitive impairments, which may underlie the accelerated aging process in APN KO mice.

Methods

Mice and Human

APN KO mice (B6;129-Adipoq^{tm1Chan}/J) were purchased from the Jackson Laboratory (Maine, USA). The WT mice (C57BL/6J) were purchased from Weitong Lihua Limited Company (Beijing, China). All mice were housed in a pathogen-free facility with a 12 h light-dark cycle (lights on at 6:00 am, lights off at 6:00 pm) and ad libitum access to food and water. D-Galactose (200 mg/kg/d) was used to induce the animal model of accelerated aging. The animal experiments were approved by the Animal Care and Use Committee of the Experimental Animal Center at Shenzhen Center for Disease Control and Prevention.

Informed consent for all human samples was obtained following the Ethical Committee of Shenzhen Center for Disease Control and Prevention. Young individuals (n=17) and BMI-matched older individuals (n=15) have additional characteristics shown in Table S1. As shown by %HbA1c, all individuals were

normoglycemic. The exclusion criteria included smoking, antibiotics usage, auto-immune disease, diabetes, hypertension, and pregnancy.

SA-β-gal for frozen sections

Firstly, 15.5 month-old WT and APN KO mice were deeply anesthetized with 4% chloral hydrate and perfused with PBS to remove intravascular blood. According to the mid-sagittal plane, the brains were rapidly removed from the skull and divided into two halves. The left hemi-brains were made of paraffin-embedded sections and used for immunofluorescence staining. The right hemi-brains were made frozen sections. For SA-β-gal staining, the right hemi-brains were fixed with 4% paraformaldehyde (PFA) for 2 days and dehydrated through a sucrose gradient (10% sucrose, 1 day; 20% sucrose, 1 day; 30% sucrose, 2 days). After that, 20-μm frozen sections were cut and rehydrated 3 times with PBS in a 6-well plate. SA-β-gal staining was performed using a kit (BestBio, China). Briefly, sections were immersed in a fixation solution for 15 min and subsequently washed with PBS 3 times. Then 2 ml per well of working solution of β-galactosidase with X-gal was placed, and the plate was maintained at 37℃ for 48 h. SA-β-gal positive areas were quantified by counting stained and unstained areas and expressed as the percent of SA-β-gal positive areas over the total area.

Immunofluorescence

The paraffin-embedded sections were deparaffinized by dimethyl benzene and rehydrated by graded alcohol, followed by a citric acid antigen repair buffer to unmask the epitope. After washing 3 times with PBS, the sections were blocked with blocking buffer (0.3% Triton X-100 + 3% bovine serum albumin in PBS) for 60 min, followed by primary antibodies including mouse monoclonal anti-GFAP and rabbit polyclonal anti-Iba1 overnight at 4℃. After primary antibodies incubation, the sections were labeled with fluorescent secondary antibodies as follows: Alexa Fluor 488 goat anti-mouse IgG (H+L) and Alexa Fluor 568 goat anti-rabbit IgG (H+L). DAPI (4, 6-diamidino-2-phenylindole) was used to counterstain the nuclei. The images were acquired using a confocal microscope and analyzed using ImageJ software.

RT-PCR

Total RNA was extracted using TRIZOL reagent (Invitrogen, Germany) from brain tissues of 15.5 month-old WT and APN KO mice. RNA integrity and concentration were verified using Nanodrop2000 (Thermo, USA). PrimeScript RT-PCR Kit (TAKARA, Japan) was used to perform reverse transcription to synthesize cDNA. Gene expression was quantified following the instructions provided with SYBR PremixEx Taq^{TM} (TAKARA, Japan). The β-actin gene was selected as a housekeeping reference. The following primers were used for quantification: EHMT1 (F) GGC ACC TTT GTC TGC GAA TAC and (R) AGA ACC GAG CGT CAA TGC AG; Baz2b (F) GCT CTA GAC GTC AGG CTT GTT and (R) TTC ACA CCG CTG GTC TTG TT; β-actin (F) TCC GGC TCA GAA CTA CAG TGT AAT and (R) TGC GGC GTT TTC ATG GT. The RT-PCR procedure was performed as the following amplification conditions: pre-denaturation at 95℃ for 2 min; 40 cycles including denaturation at 95℃ for 5 s, annealing at 54℃ for 30 s and extension at 70℃ for 34 s. Finally, the $2^{-\Delta\Delta Ct}$ method was used to analyze gene expression.

Human plasma cytokine assays

Human plasma cytokine levels of IL-1β, IL-2, IL-6, IL-8, IFN-γ, TNF-α, IL-4, IL-10, IL-12p70, and IL-13 were measured by the Meso Scale Discovery (MSD) according to the manufacturer's protocol. Briefly, plasma samples were added to the plate and incubated at room temperature. After 2 h incubation, the plate was washed 3 times before the addition of detection antibodies and read using a MESO QuickPlex SQ120. The data were analyzed using the MSD Discovery Workbench software v.4.0.

ELISA

The levels of human APN, mouse APN, inflammatory cytokines including IL-1β, IL-6, TNF-α, MCP-1, IFN-γ, IL-18, IL-4, IL-10, IL-13, and TGF-β2 were measured using ELISA kits from Elascience according to the manufacture's protocol. Dopamine signaling including dopamine (DA) and serotonin (5-HT) was detected using ELISA kits from Nanjing Jiancheng Bioengineering Institute.

Mitochondrial function and oxidative damage

Mitochondrial function was assessed by measuring ATP production using commercially available kit (Beyotime, China) according to the manufacture's protocol. Oxidative damage was assessed by measuring lipid peroxidation and GSH content. The lipid peroxidation was determined using an MDA assay kit (Beyotime, China), and the GSH level was determined using the GSH assay kit (Nanjing Jiancheng Bioengineering Institute, China).

Cell culture

The mouse microglia cell line (BV2) was purchased from the Cell Bank of the Chinese Academy of Sciences (Beijing, China). The BV2 cells were cultured in 6-well plates with DMEM/F12 supplemented with 10% FBS and incubated at 37℃ in an atmosphere of 5% CO_2. For senescence induction, BV2 cells were treated with 100 nM rotenone (Rot) or 10 nM antimycin A (Anti A) for 5 days. Aged BV2 cells were treated with 5μM AdipoRon.

Primary microglia culture was performed according to a method previously developed [13]. Briefly, brain tissues from postnatal (day 1-3) APN KO mice and WT mice were isolated, cut into tiny pieces, and followed by digestion with trypsin for 15 min. Dissociated tissues mixed with glia were then plated into T-25 culture flasks containing DMEM/F12 with 10% fetal bovine serum, GlutaMAX (Invitrogen), and 1% penicillin/streptomycin. After culture in a 5% CO_2/37℃ incubator for 14 days, the flasks were shaken at 220 rpm for 4 h at 37℃ to harvest the primary microglia. After that, the microglia were plated in 6-well plates at a density of 5 x 10^5 cells per well for inducing senescence and 10μM Cpd-60 treatment.

Flow Cytometry

Flow cytometry was used to measure the intracellular ROS level. In brief, the aged BV2 cells with or without AdipoRon treatment were incubated using 10μM 2', 7'-dichlorofluorescein diacetate (DCFH-DA,

Sigma, USA) for 30 min at 37°C. Then cells were washed 3 times with PBS and collected for analysis through BD Accuri C6 Plus (BD Bioscience, USA).

Western blot

Protein samples from animals or cells were extracted with RIPA lysis solution containing protease and phosphatase inhibitor (Thermo, USA) and then separated by SDS-PAGE and transferred to PVDF membrane. The membrane was blocked with 5% nonfat milk for 1 h at room temperature and subsequently incubated with primary antibody and secondary antibody. The protein level was measured using the Pierce^{TM} ECL Western Blotting Substrate kit (Thermo, USA) and quantified using ImageJ software.

Mouse Behavioural Assays

Open field and elevated plus-maze tests were used to assess experimental mice's anxiety-like behavior. A fear conditioning test was used to assess cognitive impairment.

Open field test

The open field consisted of a plexiglas box (50 x 50 x 40 cm). The bottom of this apparatus was divided into 16 equal squares. The inner 25 x 25 cm area was defined as central; the remaining regions were defined as peripheral areas. Mice were allowed to freely explore the device for 5 min. The device was cleaned with 75% ethanol and allowed to dry completely between each trial. Total distance traveled and the time spent in the center were recorded by Xeye software (Biowill, Shanghai, China).

Elevated-plus maze test

The elevated plus-maze was comprised of two open and two closed arms extended out from a central platform. Each mouse was individually placed in the center area and allowed to explore the device for 5 min. The time and movement of experiment mice were recorded by Xeye software (Biowill, Shanghai, China). The time spent in the open arms was calculated to assess anxiety-like behavior.

Fearing condition test

Fear conditioning was performed according to the previously described [40]. The apparatus consisted of an acrylic chamber (25 × 25 × 25 cm) equipped with a stainless-steel grid floor. On the training day, mice were first allowed to freely explore the chamber for 6 min in the absence of any other behaviorally relevant stimulus. After that, mice received 3 paired presentations of a 30 s, 4 kHz, 80 dB auditory cue (CS) co-terminating with a 2 s, 0.5 mA scrambled footshock (US), followed by the addition of 2 min of free exploration without tone or shock stimuli. Each inter-trial interval was 2 min. The chamber was cleaned with 75% ethanol between sessions to avoid residue from the previous session. On the second day, mice were placed into the original chamber to assess contextual memory. The experimental animals were allowed to explore the chamber for 8 minutes without tone or shock stimuli. On the third day, the

cued memory was tested in a novel context in which different odorants and shapes of the chamber. After a brief baseline period with no tone, the 80 dB tone sounded for 30 s at trial time points 120 s, 270 s, and 420 s. Then mice received the addition of 2 min freely exploration without any tone. The movement of mice was recorded and analyzed using the video tracking system (Biowill, Shanghai, China)

Statistical Analysis

The data was presented as the mean±SEM and analyzed using GraphPad Prism 8.0 statistical software (GraphPad Software, Inc., La Jolla, CA, USA). A two-tailed unpaired Student's test was applied to compare two groups statistically. Simultaneously, One-way analysis of variance (ANOVA) was employed to determine the statistical significance of differences among groups and follow Dunnett's multiple comparison test. A probability value of $p^{*} < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.001$ and $p^{****} < 0.0001$ was considered statistically significant.

Results

APN correlates with age, and its deficiency accelerated brain aging in mice

Initially, we examined the correlation between APN and aging by measuring APN level changes in the aged subjects (human/mice). Intrestingly declined levels of APN were detected in the aged individuals (Table 1) and experimental mice (Fig. 1a-c). Concurrent to the plasma, decreased APN levels were also observed in the brain tissue of aged mice (Fig. 1d). To further validate the correlation between APN and aging, APN KO mice were employed to measure aging-associated senescence markers, including β-galactosidase, p16, and p21. Significantly higher expressions of SA-β-gal, as well as p16 and p21 (hallmark to senescence), were detected in the brain of the old APNKO mice (Fig. 1e-j). Besides, significantly decreased levels of dopamine synthesizing enzyme (DA) and serotonin (5-HT) were detected (Fig. 1f-g), as have been associated with aging previously [2, 14]. EHMT1 and Baz2b expression were measured (Fig. 1i-j) to further validate these findings, which were deemed as conserved epigenetic regulators preventing healthy aging [39]. Aged APN KO mice exhibited dramatically higher expression of these two factors than age-matched WT mice (Fig. 1h-i).

APN deficiency correlated with anxiety and cognitive impairment in mice in aged subjects

As age is accompanied by cognitive decline and deterioration of emotional function[26], we examined the anxiety-like behavior of the APN KO mice. As shown in the figure (Fig. 2), APN KO mice of 10 months were more anxious than the 8 months. Also, both wild-type and APN KO mice showed high freezing time during the training session in a fear conditioning test, whereas APN KO mice displayed reduced freezing time during contextual and cue-induced memory tasks, indicating that APN KO deficiency could accelerate aged related cognitive dysfunction. Overall, our data strongly supported that APN deficiency could accelerate brain aging.

APN level correlated with aging-related inflammation and its deficiency enhanced neuroinflammation in mice

Chronic inflammation is one of the important characteristics of aging that is usually accompanied by accelerating proinflammatory factors [27]. The notion was then validated hereby increased proinflammatory cytokines in the aged human population (Fig. 3a). Comparatively, the imbalance between pro and anti-inflammatory cytokines was more obvious in females. The correlation between APN level and different inflammatory cytokines in female samples was further examined. Notably, APN level was negatively correlated with IL-2 and IL-6 but positively correlated with IL-13 (Fig. 3b), indicating the APN level association with age-related inflammation disorder.

Next, we sought to determine the neuroinflammatory changes in aged APN KO mice. Initially, cytokines were measured in the brain of the APN KO and WT mice (6 and 15.5 month-old) (Fig. 4). Dysregulated levels of cytokines were detected in aged mice, which were further accelerated with aging. A significantly increased level of proinflammatory cytokines was found in the brain of 15.5 month-old APN KO mice as compared with the age-matched WT mice. Both glial cells makers were (IBA-1/GFAP) increased in the brain of the APN KO mice, indicating the APN deficiency could accelerate glial activation (Fig. 4k-l). Furthermore, significantly increased expression of NLRP3, NF-kB, HO-1, and caspase 1 while decreased NRF2 expression were found, validating neuroinflammatory changes in the brain of the APN KO mice (Fig. 5). Collectively, these changes indicated that APN deficiency could facilitate the shifting of pro-and anti-inflammatory balance to the more proinflammatory statute in aged conditions.

APN deficiency enhanced mitochondrial impairment in aged mice

Mitochondrial impairment is considered the key hallmark of aging via oxidative stress and ATP level alteration [22, 24, 38]. We then performed mass spectrometry analysis and identified a total of 5392 proteins, of which 399 proteins in the APN KO group were differentially expressed. Further pathway enrichment analysis of these differentially expressed proteins was mainly enriched in inflammatory signaling pathways, such as complement activation, IL-2 signaling and IL-5 signaling (Fig. 6a). Next, the JC-1 aggregation ratio was measured (Fig. 6b) and was significantly reduced in the APN KO mice, showing that the mitochondrial membrane potential in APN KO mice is much lower than that of the normal control group. Thus these results suggested that adiponectin deletion can damage the mitochondrial membrane potential. Further, a decreased level of ATP was found in young APN KO mice compared to the control. Moreover, the MDA level was significantly increased, while GSH decreased in APN KO mice at 15.5-month compared with WT (Fig. 6c-e). Taken together, these above results indicated that APN deficiency could lead to mitochondrial impairment.

To validate the association between APN deficiency and mitochondrial impairment, we further investigated the mitochondrial physiological markers in the cortical tissues from 15.5-month WT and APN KO mice (Fig. 6f). NDUFA10 (complex I) and ATP5A (complex V) were significantly decreased in APN KO compared to WT mice, suggesting the involvement of APN deficiency in aggravating mitochondrial dysfunction. However, UQCRFS1 (complex III) was significantly increased in APN KO mice, which might

account for MDA increase. Considering different mitochondrial activities causing mitochondrial dysfunction, we further evaluated the expression of proteins involved in mitochondrial dynamics, mitochondrial biogenesis, and autophagy. Drp-1 and p62 were significantly increased while OPA-1, TFAM, PGC-1α and LC3BII decreased in aged APN KO mice, suggesting that APN deficiency increased mitochondrial fission and decreased mitochondrial fusion may lead to mitochondrial fragmentation.

APN deficiency aggravates brain aging via HDAC1 signaling

Previous studies strongly support the role of HDACs (HDAC1/2) in the expression of UPRmt (UPR mitochondrial) genes [29]. Here we also sought to determine whether mitochondrial impairment correlated with HDACs expression in APN KO condition. Initially, HDACs, including HDAC1, HDAC2 and HDAC3 expression, were examined in the cortex of the experimental mice (Fig. 7a). HDAC1 was upregulated in the APN KO old mice cortex, whereas no significant changes of HDAC2 and HDAC3 were found. Besides, H3K9me1, 2, and 3 methylations were measured with no significant changes in the methylation level of H3K9me1/2/3 found (Fig. 7a).

Next, we sought to determine the potential causal relation between HDAC1 with age and mitochondrial impairments. BV2 cells were treated with Rotenone (ROT) and Antimycin A (Anti A) to induce mitochondrial dysfunction and cell senescence [35] (Fig. 7b). ROT and Anti A significantly enhanced p16, p21, and HDAC1 expression in the BV2 cells, and these changes could be reversed by AdipoRon treatment (Fig. 7b). Further ATP and ROS changes were checked to validate mitochondrial dysfunction, which could also be decreased by AR treatment (Fig. 7c-d). Next, BV2 cells were treated with an HDAC1 inhibitor (Cpd-60) to evaluate the role of HDAC1 in ROT and Anti A-induced mitochondrial dysfunction and cell senescence. Cpd-60 treatment significantly decreased p16, p21, and HDAC1 expression induced by Anti A (Fig. 7e-f). To further examine the roles of HDAC1 in aging-related pathological processes, APN KO mice were treated with D-galactose followed by HDAC1 inhibitor (Cpd-60) treatment (Fig. 8a). D-galactose significantly enhanced HDAC1 and p16 expression, which could be reduced by Cpd-60 treatment (Fig. 8b), validating the causal relation of HDAC1 in APN deficiency-related aging and senescence. Further pathological changes were confirmed, and increased MDA and cytokines (IL-1b, IL-6 and TNF-a) levels were found in the D-galactose treated APN KO mice, which could also be reversed by HDAC1 antagonism (Fig. 8c-f). Overall, these findings indicated that APN deficiency elicited the aging process via HDAC1 signaling.

Discussion

Our findings demonstrated that APN level is negatively correlated with increased age. APN deficiency could accelerate brain aging, together with anxiety and cognitive impairments. Mechanistically, APN deficiency aggravates mitochondrial impairments via HDAC1 signaling, as evidenced by increasing HDAC1 expression in the APN KO aged mice, together with p16 and p21 upregulation and dysregulated mitochondria-associated gene expression. These above deficits could be reversed by HDAC1 inhibitor both in vivo and in vitro, validating that HDAC1 mediated mitochondrial dysfunction may underlie APN

deficient facilitated aging process. In addition, dysregulated neuroinflammation was also found in APN KO aged mice, as demonstrated by glial cell activation together with elevated proinflammatory cytokines. Interestingly, these cytokine abnormalities are closely correlated with APN changes in the age human population.

Dysfunction of immune response may parallelly occur with age and lead to chronic inflammation followed by immunosenescence[25, 30]. In agreement with previous reports, we found abnormal cytokine levels in the aged human population, which was further validated by increased senescence markers. Systemic inflammation may concurrently be followed by neuroinflammation, as the peripheral level of cytokines impairment has been evidenced along with neuroinflammation [4, 25, 34]. Interestingly, our results showed that APN KO accelerated brain aging characterized by proinflammatory states, indicating a key association between aging and neuroinflammation. As an initial defensive mechanism, the aged brain responds to stimuli (stress) by producing an exaggerated cytokine level from glial cell activations, leading to severe detriments, including prolonged sickness behaviors and cognitive impairments [30, 31]. However, it is interesting to further seek and illuminate the homeostatic imbalance of inflammation in the aged brain of APN deficient subjects.

Former studies indicate that aging cells exhibit enhanced mitochondrial DNA mutation and functional impairments in response to persistent oxidative stress[5, 6]. Moreover, imbalanced ROS production due to damaged mtDNA stimulates inflammasome (danger sensing multiprotein platform) formation [7, 12], indicating a key association between mitochondrial impairments and neuroinflammation, as evidenced by close interactions between increased inflammation and altered mitochondrial function [36]. Thus, potential inflammation suppressive factors, including adiponectin, could reduce mitochondrial damage and thus regulate inflammation. Following our previous reports [17], APN KO-aged mice displayed microglia glial cell activation together with IL-6 and TNF-a production in the hippocampal tissues on the brain. In the present study, we found dysregulated neuroinflammation together with peripheral inflammation in aged APN KO mice, demonstrating the critical role of APN linked inflammatory response in the aged brain. Interestingly, altered neuroinflammation was accompanied by mitochondrial impairment and senescence, which were further validated by mitochondria-associated inflammatory pathway impairments in mass spectrometry analysis and JC-1 ratio calculation. The exact mechanisms whereby APN KO induced glia activation still requires further clarification. As suggested in the result and others, fragmented mitochondria in APN deficiency conditions may trigger an increased secretion of proinflammatory factors, leading to neuroinflammatory response [16].

Histone deacetylases (HDACs) modify epigenetic activities and have been recently linked to mitochondrial hemostasis and longevity [8, 29]. Similarly, we have previously reported the role of HDAC1 in LPS-induced neuroinflammation and depression models [18]. APN deficiency could enhance neuroinflammation, aggravate mitochondrial dysfunctions and memory impairments, indicating that HDAC can contribute to cognitive impairments via neuroinflammation-associated mitochondrial dysfunctions. However, this phenomenon has not been linked to aging nor APN -associated activities and expression. Our results found HDAC1 upregulation in aged APNK KO mice, accompanied by enhanced

neuroinflammation and senescence, indicating a causal link between HDAC1 and mitochondrial dysfunction in APN deprived conditions. Similarly, HDAC1 level was also significantly increased in the APN KO mice upon D-galactose administration, whereas HDAC1-inhibitor reversed brain aging by decreasing mitochondrial deficits and p16, MDA and cytokines levels.

Conclusion

Overall, these findings demonstrated a partial decline of APN level during age. APN deficiency exaggerated neuroinflammation in the brain, followed by immunosenescence and cognitive impairments. Mechanistically, APN deficiency in aged subjects increased neuroinflammation associated-mitochondrial impairments via HDAC1 signaling in the brain.

Abbreviations

Histone deacetylases (HDACs), mtDNA: Mitochondrial DNA, Aβ: β-amyloid; APN: Adiponectin, MMSE: Mini-Mental State Examination, CDR: Clinical Dementia Rating, AD: Alzheimer's disease, NFTs: neurofibrillary tangles, T2D: AMPK: AMP-activated protein kinase, IRS: insulin receptor substrate, CNS: central nervous system, CSF: cerebrospinal fluid, AR: AdipoRon, 3-MA: 3-Methyladenine, CQ: chloroquine phosphate, baf A1: bafilomycin A1, 5xFAD*APN KO: APN-deficient 5xFAD, NOR: novel object recognition, MWM: morris water maze, DAB: diaminobenzidine, DAPI: 4, 6-diamidino-2-phenylindole, DTT: DL-Dithiothreitol, IAA: 3-Indoleacetic acid, DDA: data-dependent acquisition, DEPs: differentially expressed proteins, PPI: protein-protein interaction, ELISA: Enzyme-linked immunosorbent assay, ATGs: autophagy-related proteins, ALP: Autophagy-lysosomal pathway.

Declarations

Ethical Approval and Consent to participate

According to the protocols approved by the Animal Care and Use Committee of the Experimental Animal Center at Shenzhen Center for Disease Control and Prevention, all experimental procedures were carried out.

Consent for publication: All involved parties consented to the publication of this work

Competing interests. The authors declare no competing interests in this study

Availability of data and materials. All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests: The authors declare no competing financial interests.

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Author Contributions

Kaiwu He and Lulin Nie designed and performed the experiments. Tahir Ali analyzed data and wrote the manuscript. Zizhen Liu, Weifen Li, Kaiqin Zhang, and Ruyan Gao helped in the experiment, Jianjun Liu, Zhongliang Dai, Yongmei Xie, Zaijun Zhang, Gongping Liu, Ming Dong helped in the manuscript, experimental tools and supported the study. Zhi-Jian Yu, Xifei Yang, Shupeng Li endorsed the study, corresponding authors, reviewed and approved the manuscript, and held all the responsibilities related to this manuscript. All authors reviewed and approved the manuscript.

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Tables

Table 1 is not available with this version.

Figures

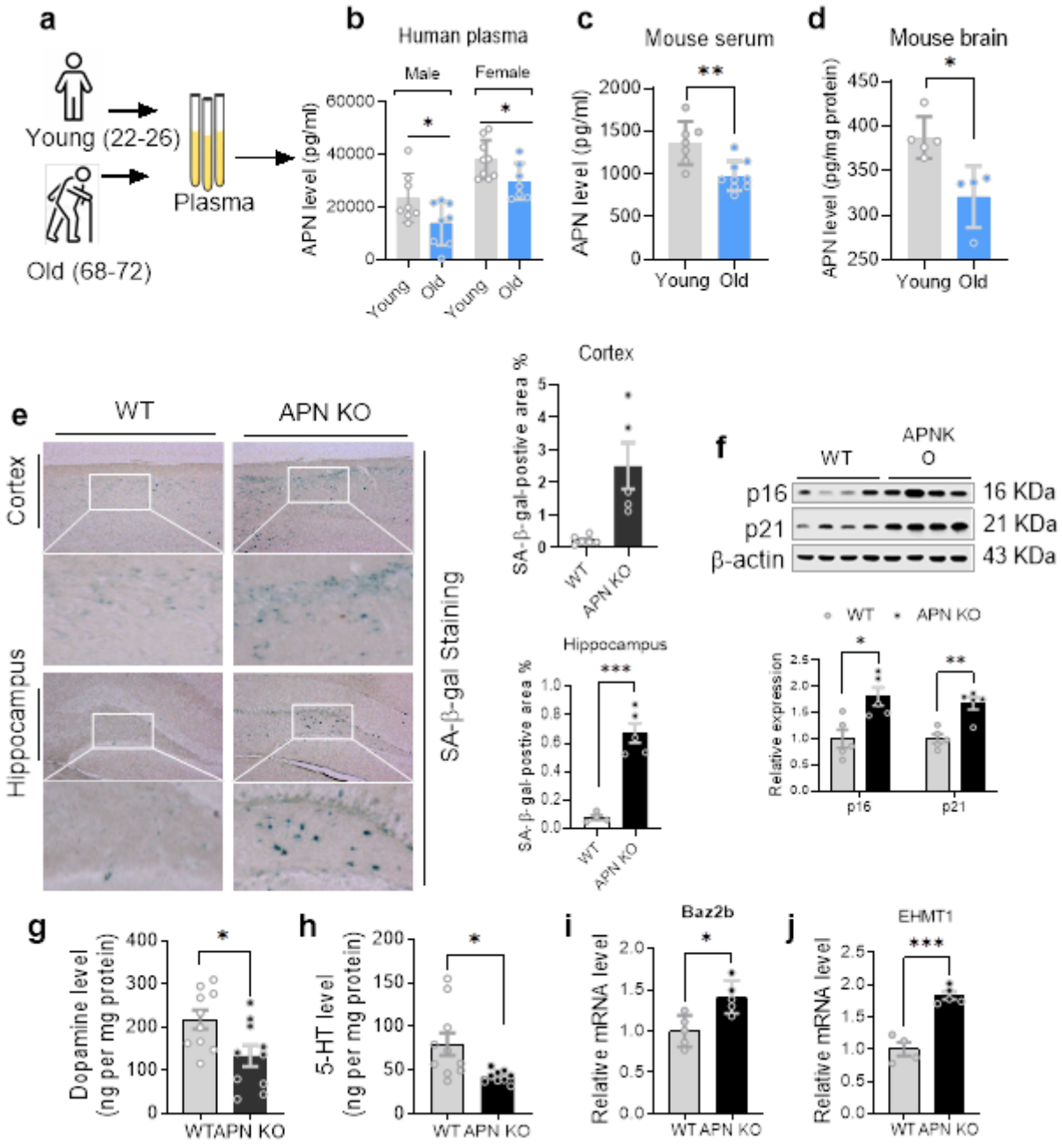

Figure 1

APN level declined in aging subjects, and its deficiency correlated immunosenescence

a-b: shows APN level changes in the plasma of aged human individuals, **c-d:** Bar graphs representing APN level changes in the plasma and cortex of the APN KO aged mice. **e:** SA-β-gal staining and quantification, **f-g:** bar graphs showing Dopamin and serotonin level, **g-h:** showing mRNA level of Baz2b and EHMT1 in the cortex tissue, **i:** representative immunoblots and bar graphs showing expression of p16 and p21, in the APN KO mice cortex. Data were expressed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

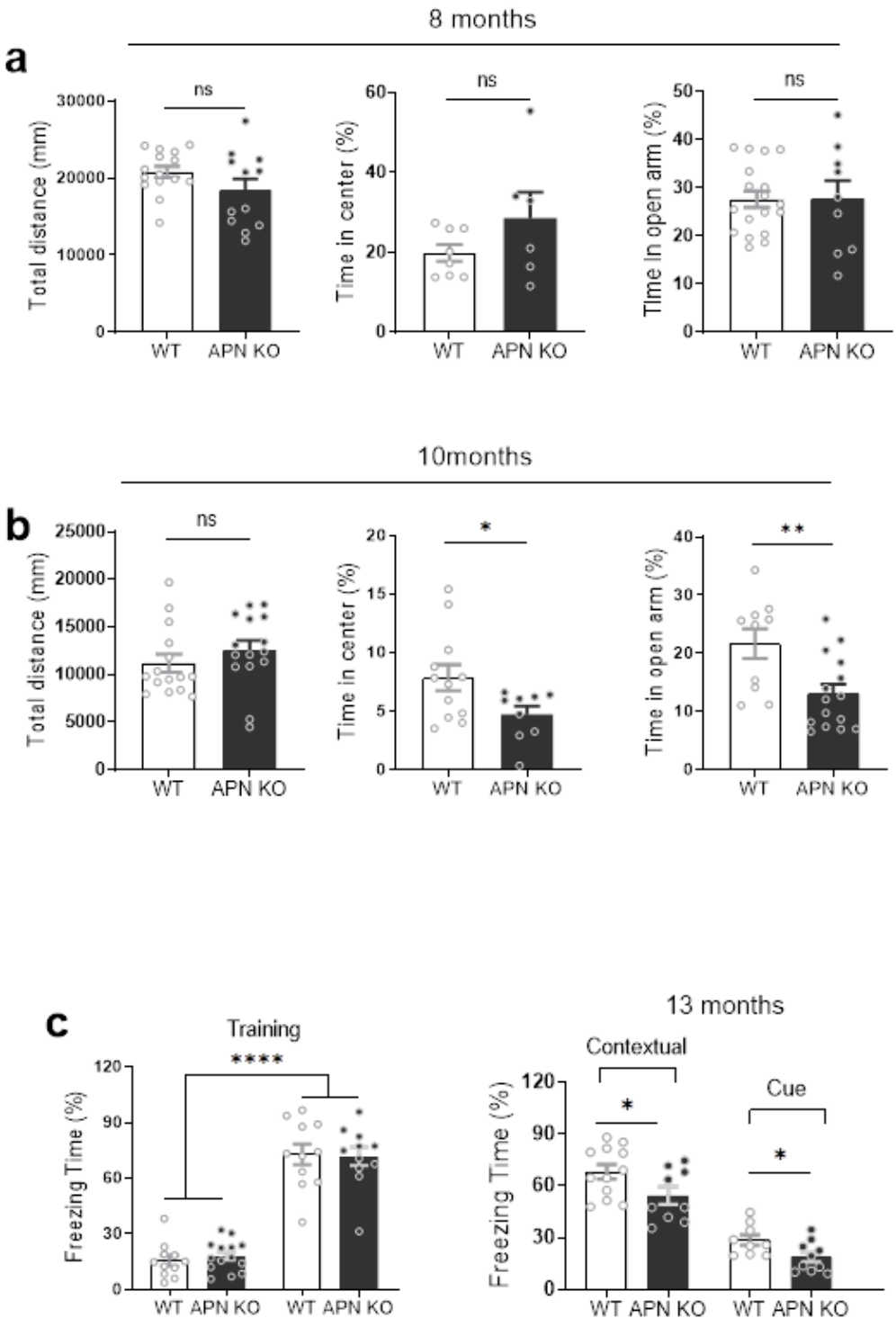

Figure 2

APN KO aged-mice displayed cognitive behaviors

a: Bar graphs show total distance, time in the center, time in open arm, **b:** total distance traveled by 10-month mice, **e:** time in the center, c: Bar graphs showing freezing time during training and test.

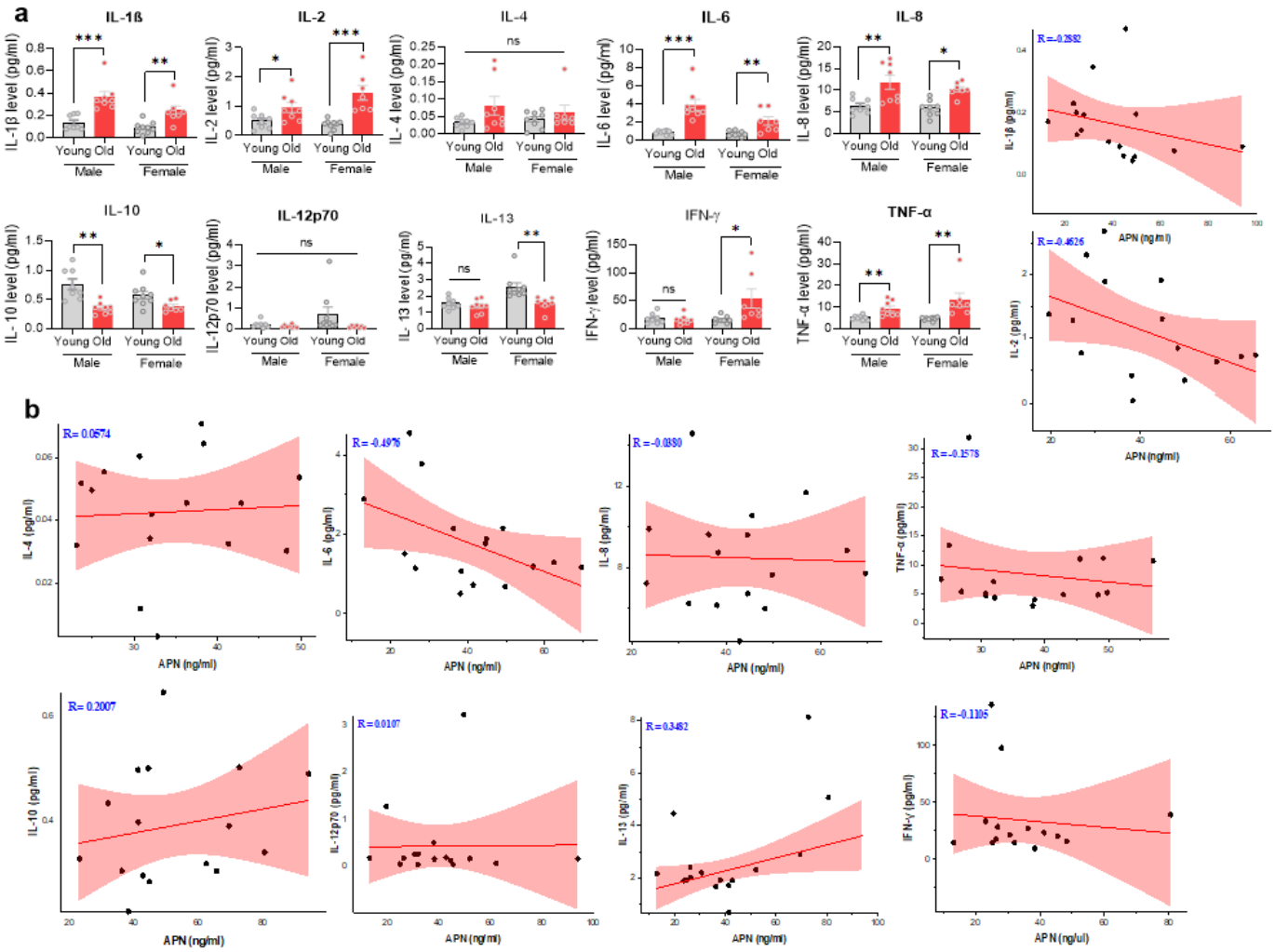

Figure 3

APN deficiency correlates with peripheral inflammation in aging subjects

a: Bar graphs showing APN level changes in the human (male/female) individuals. **b:** regression analysis, showing correlation of APN level with cytokines changes. Data were expressed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

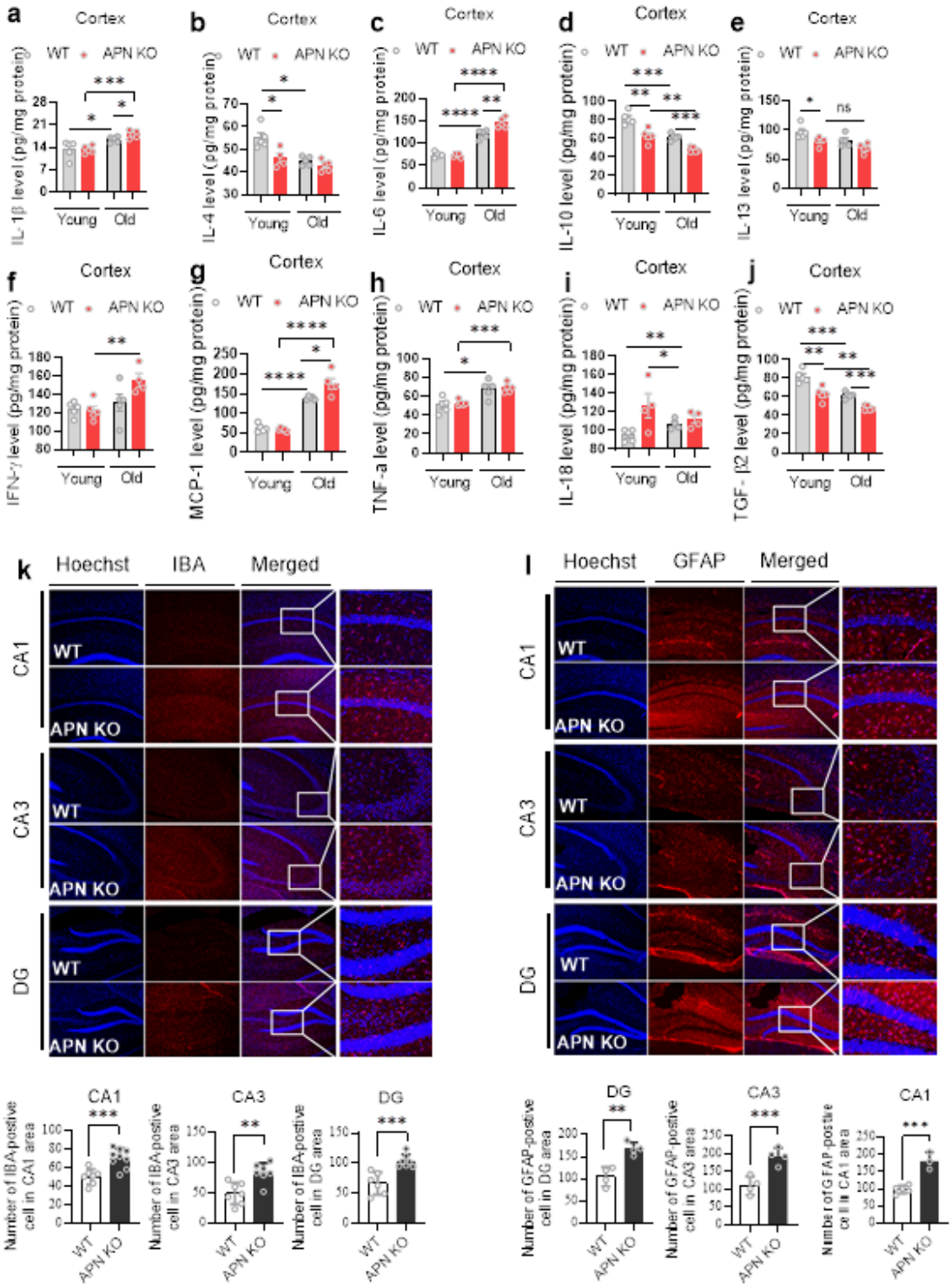

Figure 4

APN deficiency correlates with cytokine level and neuroinflammation in the APN KO mice

a-j: Shows cytokine level change in the APN KO mice cortex. **k-l:** immunostaining and bar graphs show expressional changes of IBA-1 and GFAP in the cortex of the experimental subjects. Data were expressed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

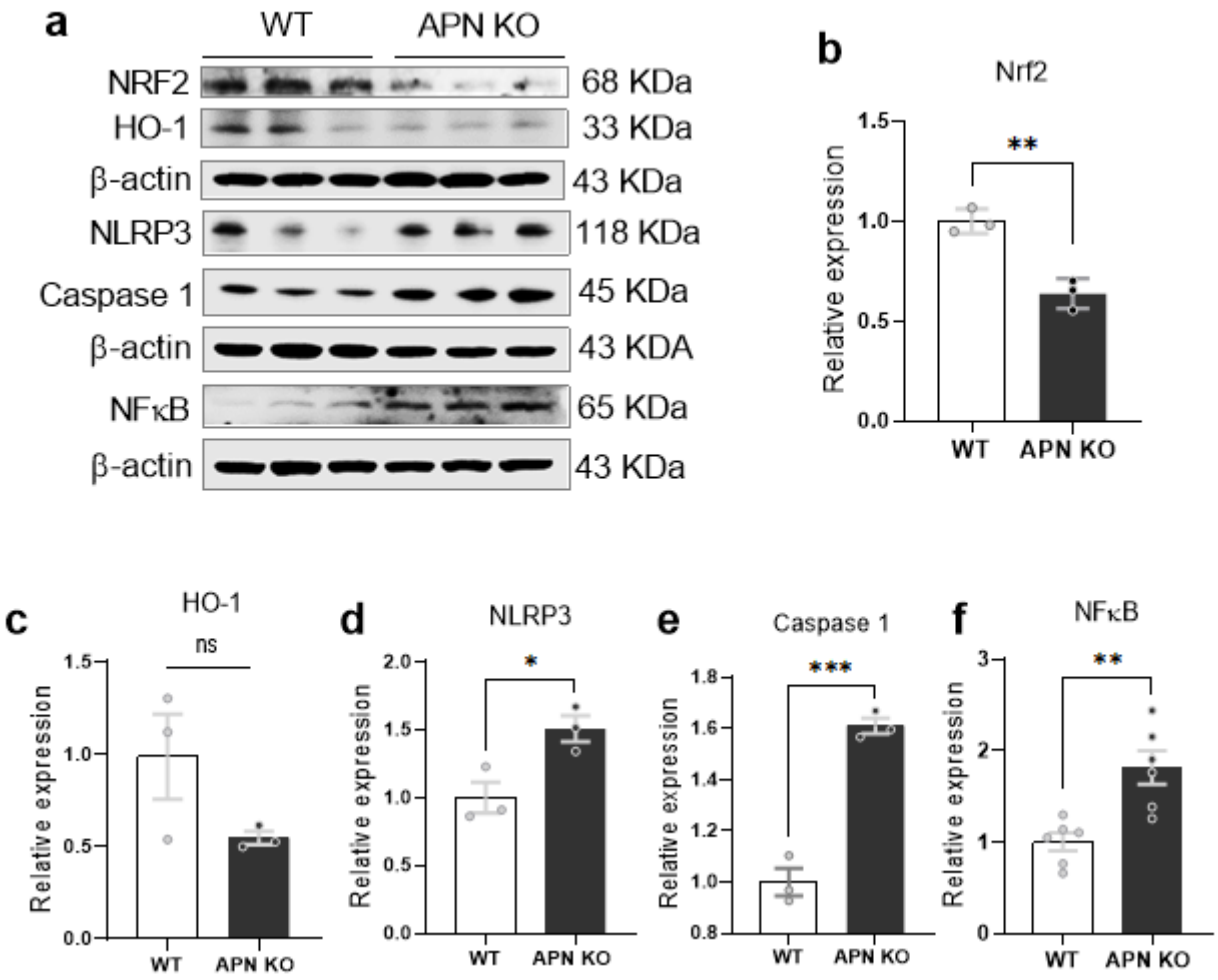

Figure 5

APN deficiency accelerates neuroinfammatory markers expression

a: Representative image of immunoblots showing NRF2, HO-1, NLRP3, Caspase 1, and NF-kB**. b, c, d, e and f**: Bar graphs showing relative expression of Nrf2, Ho-1, NLRP3, Caspase1 and NF-kB expression, respectively. Immunoblots densities were optimized by standard (GAPDH/β-actin). n=6. Data were expressed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

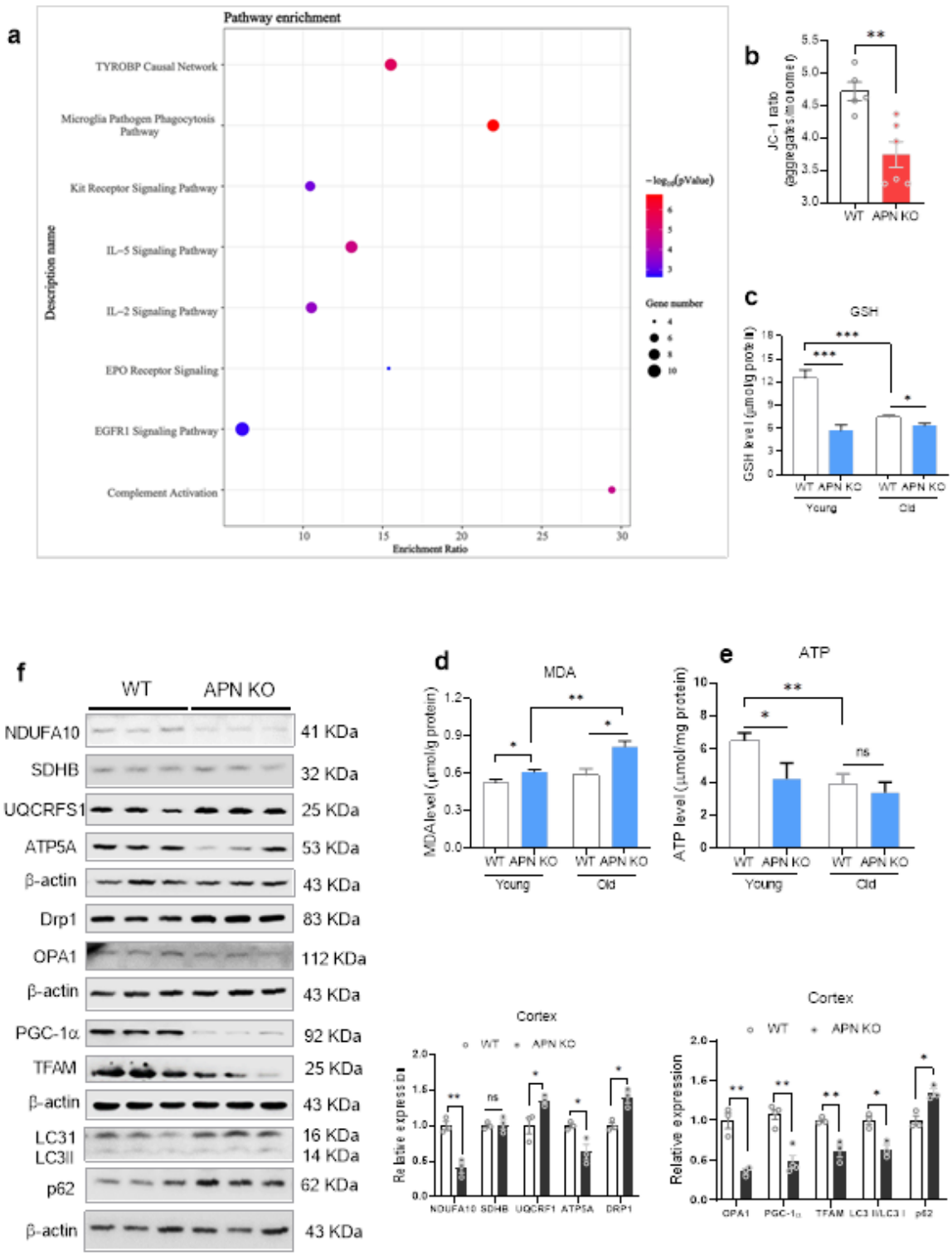

Figure 6

APN deprived aged mice displayed mitochondrial impairments

a: TMT-labeled proteomics, b: JC-1 aggregation ratio. c, d, and e: Representative bar graphs showing relative levels of ATP, MDA and GSP in the experimental subjects. f: immunoblots and their quantitative column graphs, showing expression of NDUFA10, SDHB, UQCRFS1, ATG5A, Drp-1, OPA1, PGC-1α, TFAM,

LC3BI/II, and p62. Immunoblots densities were optimized by standard (GAPDH/β-actin). n=6. Data were expressed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

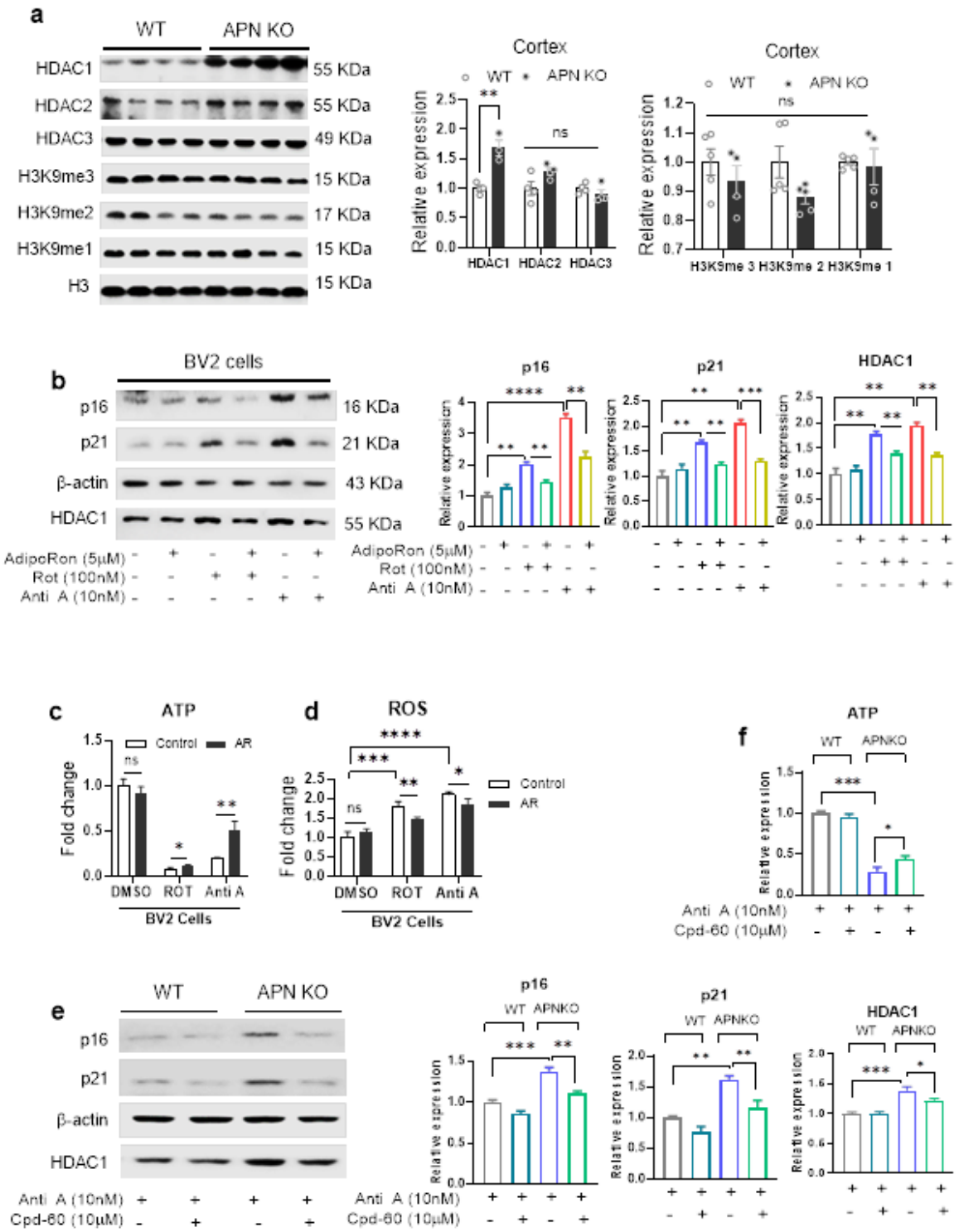

Figure 7

HDAC1 is associated with aging exaggeration in APN deficient mice

a: Immunoblots and their quantitative column graphs, showing expression of HDAC1, 2, 3, H3K9me3, 2, and 1, in the cortex of the APN KO mice. Representative immunoblots and bar graphs show p16, p21, and HDAC1 expression in the AdipoRon, Rot and Anti A treated BV2 cells. c: Bar graphs showing the level of ATP and ROS in the AdipoRon, Rot and Anti A treated BV2 cells. f: ATP level in the Anti A and Cpd-50 treated BV2 cells. e: Representative immune blots and bar graphs showing relative expression of p16, p21, and HDAC1. Immunoblots densities were optimized by standard (GAPDH/β-actin). n=6. Data were expressed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

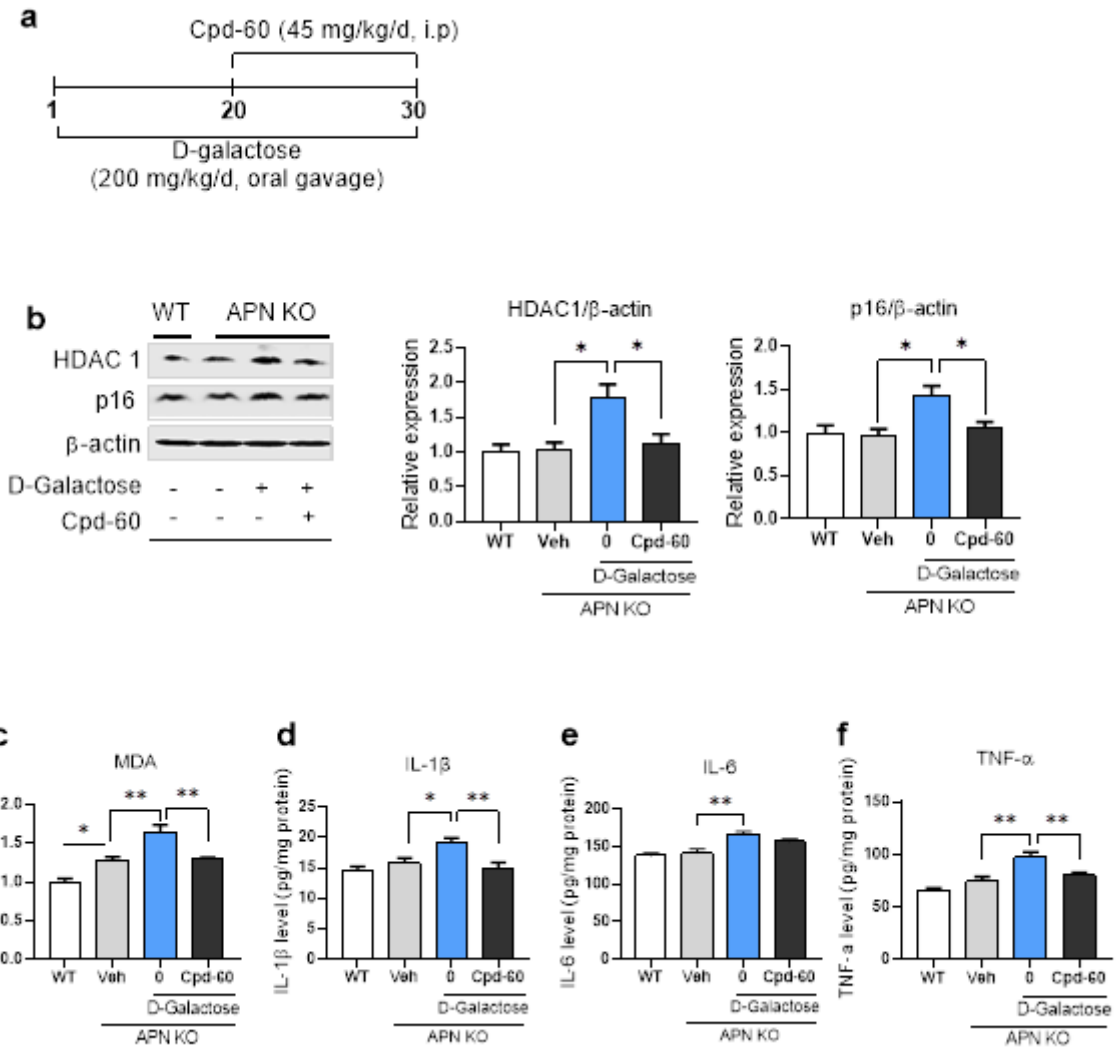

Figure 8

Cpd-60 reversed HDAC1 associated changes in the D-galactose treated APN KO mice

a: Experimental schedule/approach, b: immunoblots and bar graphs show the expression of HDAC1 and p16 in the experimental mice cortex. c, d, e and f: Representative column showing fold changes in the level of MDA, IL-1β, IL-6, TNF-α. Immunoblots densities were optimized by standard (GAPDH/β-actin). n=6. Data were expressed as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx