The BMMSCs Derived From Juvenile Macaques Have the Ability to Promote Thymus Regeneration in Aged Macaques

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Research

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Abstract

Background

The thymus gland is an important central immune organ in the human body and plays an indispensable role in the immune system. Aging thymic atrophy is critical factors leading to immune function decline and senile debilitation in aged people. Immune function decline is one of the important mechanisms of aging. Some research reports indicated that Stem cell therapy have the ability to promote tissue regeneration，but reverse thymus atrophy or promote atrophy thymus regenerate by stem cells need to be confirmed further.

Methods

The elderly macaque models were systematic screened and the thymus structure and function were evaluated by imaging and histopathology methods. The juvenile BMMSCs were injected into macaque models body by intravenous infusion. The effects of thymus tissue structure and expression of related factors were investigated by using imaging, histopathology,cell and molecule observation and measurement techniques. To explore the mechanism of juvenile BMMSC on thymus atrophy in aging rhesus macaques, the gene transcription profile of thymus tissue were sequencing and analyzed by bioinformatic methods. .

Results

Through PET-CT observation, the thymus tissue density gradually increased after treatment, CT value gradually increased, SUVmax >1;The percentage of $CD3^+$T cells in peripheral blood increased first and then decreased, $CD3^+CD4^+$T cells increased slowly, and $CD3^+CD8^+$T cells increased first, then decreased and then increased. By detecting Tregs in peripheral blood, the percentage of Tregs in peripheral blood showed a trend of decreasing first and then increasing after treatment (P< 0.05).The levels of thymosin α and thymosin Ⅱ in peripheral blood were analyzed by ELISA method. It was found that thymosin α was firstly increased and then decreased after BMSC infusion, and the levels of thymosin Ⅱ were firstly increased and then decreased.The area of thymus parenchyma increased, and the boundary between skin and medulla appeared. Some thymus tissues were regenerated and changed to normal structure.The cortical and medullary junction structure and dense thymus structure gradually appeared in the elderly treatment group.The degree of thymus tissue fibrosis was reduced, and the deposition of collagen fiber was reduced. Apoptosis cells decreased significantly in the elderly treatment group compared with the elderly group. BMMSCs inhibited the expression of genes related to aging and apoptosis.

Conclusion

Transplantation BMMSC derived from juvenile macaques can poromote thymus regeneration, reverse thymus atrophy by regulating gene transcription profiles in aged macaques.

Introduction

The world is undergoing a rapid transition to aging[1]. Aging is a ubiquitous phenomenon in the biological world, which leads to the degeneration or loss of a series of functions at the level of tissues and organs[2].An important cause of aging is a series of functional and structural changes of the immune system, and the thymus is an important central immune organ of the human body.The thymus is the site of T cell differentiation, development and maturation, and determines the effectiveness of T cell immune response[3].With the growth of age, the thymus gland will atrophy and degenerate, leading to the decline of the body's ability to fight infection, increasing the incidence of tumors and the decline of autoimmunity.Therefore, it is very necessary to study how to improve and delay the aging - related thymic degeneration changes[4].

In recent years, many studies have shown that the treatment options for delaying thymus senescence include cell adoptive therapy, gene recombination therapy[5], activated signaling pathway and bioengineering therapy[6].However, these methods have certain risks and challenges.

Mesenchymal stem cells (MSCs) are a kind of cells with multidirectional differentiation potential and self-renewal ability.Based on cell therapy, bone marrow mesenchymal stem cells have become a popular trend in cell regeneration therapy, due to their biological characteristics such as easy access, easy amplification, low immunogenicity, anti-inflammatory and immunomodulatory function[7].Many clinical and basic studies have shown that bone marrow mesenchymal stem cells are effective in the treatment of thymus senescence and may be a new method to delay or reconstruct thymus senescence[8,9].

Rhesus macaques are non-human primates, and their genome sequencing is as high as 93% similar to human genomes[10]. The research results have more reference value than other animals, and have a high degree of homology with human genetic material.

At present, most of the researches are based on rodent models, and few researches on non-human primates. At the same time, the mechanism of bone marrow mesenchymal stem cell transplantation on the improvement of thymus function in aging macaque monkeys is still unclear, and its therapeutic effect is worthy of further exploration.

Materials And Methods

Experimental animal

Kunming Institute of Zoology, Chinese Academy of Sciences [2019-032 (Section)-01] provided 5 healthy female juvenile macaques, with an average age of 3 years and body weight of 2.0-3.0 kg;10 healthy elderly female macaques with an average age of 25 years weighing 4.0-5.0 kg.All the macaques are stored in the animal room of the 920th Hospital of the PLA Joint Logistics Support Force.All the aged macaques showed senescence phenotype, dark hair color, poor mobility, obvious brain atrophy,

pulmonary fibrosis, thymus and ovary atrophy.All animal studies were approved by the Laboratory Animal Ethics Committee of the supporting unit, animal certificate number :SYXK (Military) 2012-0039.

Main reagents and antibodies

0.25% trypsin and penicillin-streptomycin solutions were purchased from Hyclone;Fetal bovine serum from ServiceBio; DMEM/F12 medium, DMSO and sodium pentobarbital were purchased from Sigma;Trizol from Invitrogen;Rhesus monkey bone marrow mesenchymal stem cell medium, osteogenic induction differentiation medium (MKRMA-90021), adipogenic induction differentiation medium (MKRMA-90031) and chondrogenic induction differentiation medium (MKRMA-90041) were all purchased from Cyagen.FITC Anti-Human CD29 (11-0299-42), FITC Anti-Human CD34 (11-0349-42), FITC Anti-Human CD90 (11-0099-42) and FITC Anti-HumanCD105 (12-1057-42) were purchased from Ebioscience;Cell staining buffer (420201), FITC anti-human CD3 (556611), PE anti-human CD8A (301007), and APC anti-human staining bufferCD4 (317415), true-nuclear Transcription Factor Buffer Set (424401), Cell staining Buffer (420201), Alexa Fluor® 647Anti-human Foxp3 (320113), FITC anti-human CD4 (317415), PE anti-human CD25 (356103), PE Mouse IGG1, PE Mouse IGG1, PE Mouse IGG1κ ISOTYPE CTRL (400111), Alexa Fluor® 647 Mouse IGG1, and κ ISOTYPE CTRL (320113) were all purchased from Biolegend;Monkey thymosin α ELISA kit (MM-232601) and monkey thymosin poietin ELISA kit (MM-234201) were purchased from ELISA;P53 Antibody (2527S), P21 Antibody (2947S), and Sirtuin3 Antibody (2627S) were purchased from CST;Sirtuin1 Antibody (DF6033) purchased from Affinity;Bax Antibody (NBP2-29468) and Cytokeratin 5 Antibody (NBP2-22194) were purchased from Novus;Bcl-2 Antibody (ORB304697), purchased from Biorbyt;Caspase3 Antibody (19677-1-AP), purchased from Proteintech;Cytokeratin 8 Antibody (LS-C383073), purchased from LSBio;8F-FDG was purchased from the First Affiliated Hospital of Kunming Medical University.

Experimental protocols

Preparation and identification of BMMSCs from young macaques

Bone marrow of healthy young female macaques with an average age of 3 years was collected by bone marrow puncture under aseptic conditions. BMMSCs were isolated and purified by differential attachment method and passage culture method. BMMSCs were obtained by four passage amplification times.The morphology and growth status of BMSCs were observed under inverted phase contrast microscope. The positive expression rates of CD29, CD34, CD90 and CD105 cells were detected by flow cytometry. BMMSCs were induced to differentiate into adipoblast, osteoblastic and chondroblast cells in vitro, and the differentiation ability was detected.

Animal grouping and BMMSCs transplantation treatment of macaque

The 15 rhesus macaques had no abnormal changes after 1 week of normal feeding and were randomly divided into groups, namely 5 in the juvenile group (n=5), 4 in the elderly group (n=4) and 6 in the elderly treatment group (n=6). In the elderly treatment group, BMMSCs were transplanted via femoral vein at a

dose of 1×10^7cells/kg, once every other day, for 3 consecutive infusions; the juvenile group and the elderly group were infused with equal volume of normal saline at the same time. Routine feeding was carried out, and the macaques of each group were sacrificed 6 months after the last transplantation, and the materials were collected.

Imaging observation of thymus

PET-CT scans were performed on macaques in the juvenile group, macaques in the elderly treatment group before treatment, 3 months after treatment in the elderly treatment group, and 6 months after treatment in the elderly treatment group. Rhesus macaques were fasted for at least 6 hours before PET-CT scan, blood glucose measured after anesthesia <8 mmol/L, intravenous injection of 18F-FDG at a dose of 0.3 mCi/kg (by body weight), and rest for 60 to 90 minutes, PET- CT scan. CT uses conventional whole-body spiral scanning, tube voltage 120kV, tube current 260 mA, pitch 0.561, rotation speed 0.5 s/cycle, layer thickness 3.75 mm, interval 3.75 mm, matrix 512 × 512, FOV 50 cm × 50 cm; subsequent PET scan , Each bed is collected for 2.5 min.

Evaluation of thymus function in macaques

Periphereal blood was collected from femoral vein of macaques before treatment and 30, 60 and 90 days after treatment. Flow cytometry was used to detect the output levels of T cells and regulatory T cells in peripheral blood of rhesus monkeys, and ELISA was used to analyze the levels of simian thymosin α and simian thymosin in peripheral blood of rhesus macaques.

Evaluation of thymus tissue structure in macaques

3% pentobarbital sodium (1kg/ml) was used to anesthetize and kill the macaque monkeys in each group, and the thymus tissue was obtained.After the thymus tissue was removed, the thymus tissue was fixed with 4% PFA solution for 24h.The thymus tissue was dehydrated and paraffin-embedded in sections with a thickness of 4μm.He staining was used to observe the changes of thymus tissue structure, immunofluorescence staining was used to observe the changes of thymus cortex and medulla structure, Masson staining was used to observe the changes of collagen fiber deposition , TUNEL staining was used to observe the apoptosis of thymus cells and gene expression was observed by immunohistochemistry staining .

Transcriptomics sequencing analysis of thymus tissue gene

For sample collection and preparation, first perform RNA extraction and detection, and secondly perform library construction and quality inspection. After the library is constructed, use Qubit2.0 Fluorometer for preliminary quantification, dilute the library to 1.5ng/ul, and then use agilent 2100 bioanalyzer the insert size of the library is tested. After the insert size meets the expectation, qRT-PCR will accurately quantify the effective concentration of the library (the effective concentration of the library is higher than 2nM) to ensure the quality of the library and perform online sequencing. The main links of data analysis include data accusation, sequence alignment to reference genome, new transcript prediction, gene expression

level quantification and differential expression analysis. Use DESeq2R software (1.16.1) to perform differential expression analysis between the two comparison sets (Two biological replicates per group). Through DESeq2, genes with adjusted P values <0.05 were assigned as differentially expressed genes. The corrected P value and |log2foldchange| are used as a threshold for significant differential expression, and a linear model is used to screen out aging-related genes.

Statistical analysis

All data are analyzed by SPSS 21.0 statistical analysis software. The statistical results are expressed as `x±s. The statistical comparison of differences between groups is performed by one-way ANOVA, P <0.05 is considered significant.

Results

Preparation and identification of juvenile BMMSCs

Primary BMMSCs showed colony growth, and the cells were evenly dispersed on the bottom of the bottle. When the cell fusion rate reaches 80%~90%, it will be subcultured and expanded. With the subculture, the P4 generation cells began to become uniform in morphology, with less impurities, and grew in a long spindle shape, fibroblast-like growth, high cell density, growth in a whirlpool shape, and good growth status (Fig. 1A).

Flow cytometry analysis of P4 generation BMMSCs surface markers CD29, CD34, CD90 and CD105, the positive expression ratios were 98%, 0.99%, 98.8% and 99.8%, respectively, in line with the phenotypic characteristics of BMMSCs(Fig. 1B).

BMMSCS obtained by differential adherence method and subculture method are long spindle-shaped and fibroblast-like; after in vitro lipogenic induction and staining with Oil Red O, the cell morphology becomes round and red-stained lipid droplets can be seen in the cell. Rhesus monkey BMMSCs were induced in vitro to form osteoblasts. After staining with alizarin red, a large number of obvious dark red calcareous nodules were seen; Rhesus monkey BMMSCs were induced to form cartilage in vitro and stained with acian blue, the morphological changes were obvious, and the proteoglycan synthesis in cartilage tissue , visible blue (Fig. 1C).

Thymus formal and functional changes after MSC treated by PET-CT imaging observation

The PET-CT of the thymus in the juvenile group and the elderly treatment group showed that the thymus position was normal. The density of the thymus in the juvenile group was as high as 63HU, and SUVmax>1; in the elderly group, most of the thymus was replaced by adipose tissue before treatment, only a few fine fiber cord-like structures were seen, the density was the same as the adipose tissue, PET SUVmax<1; March and June after treatment The density of thymus tissue gradually increased, the CT value gradually increased, reaching 68HU, PET SUVmax>1Fig.2;Table1.

Changes in peripheral blood T cell and regulatory T cell output levels

By flow cytometry, the percentage of $CD3^+$T cells in the peripheral blood of macaques in the elderly treatment group increased at first and then decreased, and increased significantly at 30 d and 60 d compared with before treatment (P<0.05), but there was no significant difference at 90 d (P>0.05)(Fig. 3A);The percentage of $CD3^+CD4^+$T cells in peripheral blood increased slowly, and there was no significant difference between before treatment and 30,60,90 days after treatment (P>0.05);The percentage of $CD3^+CD8^+$T cells in peripheral blood increased first, then decreased and then increased (Fig. 3B).

The percentage of regulatory T cells in peripheral blood showed a decreasing trend, and there was significant difference between before and after 30, 60 and 90 days of treatment (P<0.05) (Fig. 3C).

Changes of thymosin α and simian thymosin □ in peripheral blood of macaques

The levels of thymosin α were firstly increased and then decreased after BMMSCs infusion, and were significantly increased after 30 days of treatment compared with before treatment (P<0.01);After 60 d and 90 d of treatment, there was no significant difference (P>0.05) (Fig. 3d).

The levels of thymopoietin □ in macaques increased at first and then decreased, and increased at 30 and 60 days after treatment compared with before treatment, and decreased at 90 days after treatment, but there was no significant difference (P>0.05) (Fig. 3e).

Thymus tissue structural regeneration

The juvenile group (Fig.2-A) showed that the thymus tissue envelope was relatively intact, with a clear junction between the cortex and the medulla, the parenchymal area of the thymus is large and full. In the elderly group of macaques, the thymus gland was significantly atrophied and degenerated, the skin and medulla junction were blurred and the cortical area was filled with adipose tissue(Fig. 5A). In the treatment of BMMSCs, the elderly treatment group(Fig.5A) increased the parenchymal area of the thymus compared with the elderly group, the structure of the cortex and medulla area improved and clear boundaries began to appear, the thymocytes increased, and the fat tissue filling decreased.

By immunofluorescence staining (Fig.5B), the cortex and medulla junction structure gradually appeared in the elderly treatment group, and the thymus structure was dense. The expression of CK5 was highest in the juvenile group and lowest in the elderly group. The expression in the elderly treatment group was significantly higher than that in the elderly group (P<0.05); the expression of CK8 was the highest in the juvenile group and the lowest in the elderly group. The expression in the elderly treatment group was significantly higher than that in the elderly group (P<0.01) The expression of CK5+CK8 was highest in the juvenile group, and the lowest in the elderly group. The expression of CK5 in the elderly treatment group was significantly higher than that in the elderly group (P<0.05), and the expression of CK8 showed an upward trend compared with the elderly group.

Masson staining of tissue sections, the juvenile group are arranged neatly and densely, the collagen fibers are blue, the collagen deposition area is small, and the degree of fibrosis is low(Fig. 5C); with the aging, the elderly group A large number of collagen fibers appeared in the thymus tissue, the arrangement was disordered, the collagen deposition area increased, and the fibrosis was obvious(Fig.5C); the collagen fiber deposition area of the elderly treatment group was significantly lower than that of the elderly group, and the degree of thymic fibrosis was significantly reduced(Fig.5C).

Tunel staining of rhesus macaque thymus tissue(Fig.5D)showed that the juvenile group had the least thymocyte apoptosis, the elderly group had the most thymocyte apoptosis, and the elderly treatment group had significantly fewer apoptotic cells than the elderly group.

Changes in protein expression of aging and apoptosis related genes

The expression of P21 gene was the lowest in the young group and the highest in the elderly group. The elderly treatment group was significantly lower than the elderly group (P<0.05). The expression of P53 gene was the lowest in the young group and the highest in the elderly group. The elderly treatment group was significantly lower than that of the elderly group (P>0.05). Sirt1 gene expression was highest in the juvenile group and lowest in the elderly group. The elderly treatment group had a higher trend than the elderly group (P>0.05). Sirt3 gene expression in the elderly group was lower than that in the young group (P<0.05), and the elderly treatment group had a higher trend than the elderly group (P>0.05). Caspase3 and Bax gene expression was the lowest in the young group and highest in the elderly group. The elderly treatment group was significantly lower than the elderly group (P<0.01); the Bcl-2 gene expression was the lowest in the elderly group, and the elderly treatment group had higher expression than the elderly group (P<0.05). And slightly higher than the juvenile group(Fig.6A).

Changes of gene Transcription felies after MSC treated

Using linear model analysis, a total of 312 differentially expressed genes related to thymic tissue aging were detected based on P<0.05, among which 305 genes were up-regulated with age. 7 genes were down-regulated with age. Based on thymic aging-related genes, 3D PCA analysis was performed on the expression matrix results of thymus tissues of various ages, and it was found that the overall transcriptome characteristics of the elderly treatment group (dark blue) had a very obvious tendency compared with the elderly group (purple). The trend of the direction(Fig.7A) .At the same time, cluster analysis was used to discover the changes in thymic senescence-related gene expression before and after BMSC transplantation (Fig.7B). And through trajectory analysis, we found the trajectory of thymic aging-related genes(Fig.7C and D). The results showed that, compared with the elderly group, the thymus expression characteristics after BMSC transplantation tended to be the expression characteristics of the young rhesus monkey thymus. The GO enrichment analysis of genes that were up-regulated with aging and decreased after treatment, according to the -log P value corresponding to each item, sorted from large to small to get a total of 17 items (Fig.7C). Through GO enrichment analysis, we found that genes that are up-regulated with aging and decreased after treatment are mainly enriched in cytokine-cytokine receptor interaction (Cytokine-cytokine receptor interaction), nephron tubule formation (Nephron tubule formation),

and synaptic signaling (Synaptic signaling), positive regulation of ion transport, regulation of osteoclast differentiation (Regulation of Osteoclast Differentiation) and other pathways.

Discussion

Thymus glands gradually shrink and degenerate with age, and aging and immune function degradation are inseparable. In this study, the anatomical structure and function of thymus tissue were analyzed by PET-CT imaging. We found that the light transmittance of thymus tissue increased significantly, and SUVmax showed a gradual increase trend. It has been reported in the literature that PET-CT can be used to track the transplantation of differentiated BMMSCs and the model of myocardial infarction to restore cardiac function[11]. Therefore, PET-CT is an important tool for tracking the anatomical structure and function of thymus tissue.

T cells are a kind of extremely active cell population derived from hematopoietic stem cells and mature in the thymus, accounting for about 60% of peripheral blood lymphocytes.According to our results, $CD3^+$T cells, $CD3^+CD4^+$T cells and $CD3^+CD8^+$T cells were increased in naturally aging macaque monkeys after infusion of juvenile BMMSCs compared with before treatment, suggesting that BMSCs can improve thymus output function [12] and increase thymus T cell output. Observed 90 days after the treatment of $CD3^+$ T cells proportion is on the decline, guess may be associated with the regulation of extracellular vesicles, and no statistical differences compared with before treatment, cell function has the timeliness, not once and for all, have reference to the future clinical cell therapy, cell therapy should have certain treatment, as well as the dynamic observation.

The results of this experiment showed that after BMSC infusion, the overall level of T cells of immune cells increased, and Tregs decreased.As can be seen from the results, Tregs showed a downward trend on the 30 and 60 days after treatment, reached the lowest point on the 60th day, and showed an inflection point and an upward trend on the 90 day. We speculate that there is a direct relationship between the number of cells and the time of infusion.It has been reported that in vitro co-culture of MSC and immune cells, when the number of MSC cells exceeds a certain amount, the number of Tregs is inhibited, and when the number of MSC cells is below a certain number, the proliferation of Tregs can be promoted.

Thymus epithelial cells and their secreted thymosin and thymosin are the main components of the thymic microenvironment, which allow thymocytes to migrate and interact with each other, helping thymocytes to develop and mature, and both of them can promote the differentiation of thymocytes[13].However, cytokines secreted by thymus epithelial cells and hormone-mediated signal transduction showed a decreasing trend during aging.The experimental results showed that thymosin α and thymosin were increased in the thymus of aging macaques after the infusion of bone marrow mesenchymal stem cells, which proved that BMSCs can improve the secretion function of thymus [14] and enhance the immune function [15, 16], and play an important role in maintaining the homeostasis of the microenvironment of thymus.

Zhan et al.[17] used human adipose-derived mesenchymal stem cells to treat thymus damage during chemotherapy and found that human adipose-derived mesenchymal stem cells expanded the area of the thymus damaged by chemotherapy and promoted the recovery of the thymus. This experiment found that after treatment with BMMSCs, compared with the elderly group, the senile rhesus macaques in the elderly treatment group increased in volume, the skin and medulla structure improved and began to appear boundaries, thymocytes increased, and adipose tissue decreased; and it was conducive to the deposition of collagen fibers in the thymus tissue The improvement of thymus tissue reduces the degree of fibrosis. We infer that the effect of BMMSCs on the thymus is mainly through the differentiation of thymocytes and the paracrine and immunomodulatory effects of BMMSCs[18], which may be related to the immunomodulatory cytokines secreted by BMMSCs, such as transformation Growth factor-β, hepatocyte growth factor, vascular endothelial growth factor, fibroblast growth factor. thereby exerting an immunomodulatory effect in the thymus and promoting the improvement of thymus tissue.

By immunofluorescence staining, it can be found that the cortex and medulla junction structure gradually appeared in the elderly treatment group, and the thymus structure was dense. The expression of CK5, CK8 and CK5+CK8 in the thymus of macaque was highest in the juvenile group, and the lowest in the elderly group. The expression in the elderly treatment group was higher than that in the elderly group. Upward trend. We can speculate that BMMSCs can improve the effect of aging thymus and gradually transform the structure of thymus to normal structure. Zhan et al.[19] analyzed CK8 and CK5 immunofluorescence and found that human adipose-derived mesenchymal stem cells can repair the damaged thymus during chemotherapy, improve the proliferation of thymic epithelial cells, and can restore normal in the chemically damaged thymus in mice structure.

Whether it is caused by oxidative stress or telomere, it is related to the activation of transcription factors and the tumor suppressor gene P53 and its downstream gene P21[20]. We observed that the expression of P21 and P53 gene protein was the lowest in the young group and the highest in the elderly group. The elderly treatment group was significantly lower than the elderly group (P<0.05). Therefore, we speculate that the reduction of P53 and P21 can promote stem cell division, accelerate cell growth, and improve thymic senescence[21].

Sirt1 is related to multiple signal transduction pathways, and participates in neuroprotection, glucose and lipid metabolism, cell senescence and cell apoptosis and other reactions, thereby exerting gene regulation. Sirt3 is a mitochondrial deacetylase, which is abundantly expressed in the heart, brain and adipose tissue, and plays different roles in metabolic regulation, cell proliferation and gene stability[22]. Sirt1 and Sirt3 gene protein expression was highest in the juvenile group, and lowest in the elderly group. The elderly treatment group had a higher tendency than the elderly group. In this study, the expression of Sirt1 and Sirt3 increased after BMMSCs transplantation treatment. It is speculated that Sirt1 and Sirt3 promote the repair of thymic tissue in the elderly, and Sirt1 and Sirt3 play an important role in maintaining the youthful state of thymus tissue[23].

In this study, the protein expression of apoptosis-related genes Caspase3 and Bax gene was the highest in the elderly group, significantly decreased after treatment in the elderly group, and the lowest in the young group. The protein that inhibits the expression of apoptosis gene Bcl-2 was the lowest in the elderly group, and the expression in the elderly treatment group increased after treatment, and was slightly higher than that in the young group. Secondly, the results of Tunel staining of thymus tissue showed that with age, thymocyte apoptosis increased, but after BMMSCs transplantation treatment, thymocyte apoptosis decreased. Therefore, it is speculated that BMMSCs may improve thymic tissue aging by reducing thymocyte apoptosis and aging[24, 25].

mRNA is a large class of RNA molecules, which transmit genetic information from DNA to ribosomes, serve as a template for protein synthesis and determine the amino acid sequence of the peptide chain of gene expression products. The detection of mRNA can indirectly reflect the expression of genes[26]. In this study, the expression matrix of thymus tissue was obtained through gene transcriptomics sequencing, and linear model analysis was used. Based on $P<0.05$, a total of 312 differentially expressed genes related to thymic tissue aging were detected, which increased with age 305 genes were up-regulated, and 7 genes were down-regulated with age. Based on thymic aging-related genes, 3D PCA analysis was performed on the expression matrix results of thymus tissues of various ages, and it was found that the overall transcriptome characteristics of the elderly treatment group had a very obvious trend toward the youth group compared with the elderly group. At the same time, cluster analysis was used to find the changes in thymic senescence-related gene expression before and after BMMSCs transplantation, and the trajectory of changes in thymic senescence-related genes was found through trajectory analysis. The results showed that, compared with the elderly group, the thymus expression characteristics after BMMSCs transplantation tended to be the expression characteristics of the young rhesus monkey thymus. The GO enrichment analysis of genes that were up-regulated with aging and decreased after treatment, were sorted according to the -log *P* value corresponding to each item in descending order to get a total of 17 items. Through GO enrichment analysis, we found that genes that are up-regulated with aging and decreased after treatment are mainly enriched in Cytokine-cytokine receptor interaction, Nephron tubule formation, and synaptic signaling. (Synaptic signaling), Positive regulation of ion transport, Regulation of Osteoclast Differentiation, etc. In general, after BMMSC transplantation to treat senescent macaques, the transcriptome characteristics related to aging were significantly reversed.

In summary, juvenile BMMSCs can improve the structure and function of the thymus of senile macaques. However, in the process of improving the structure and function of the thymus, which related genes and signal pathways are involved in the regulation of BMMSCs, there are still shortcomings in this study. We will establish a more comprehensive set, systematic evaluation criteria and in-depth study of molecular mechanisms.

Conclusions

1. BMMSC transplantation can promote the reconstruction of thymus structure, improve the T cell output capacity and thymus hormone production function in aging macaques.

2. BMMSC transplantation modulates aging and apoptosis-related genes and ameliorates thymus degeneration.

Abbreviations

BMMSCs: Bone marrow mesenchymal stem cells; FOXP3: Recombinant Forkhead Box P3; P53: Tumor Protein 53; P21: Tumor Protein21; MSCs: Mesenchymal stem cells;18F-FDG: β-2-[18 F]-Fluoro-2-deoxy-D-glucose;FBS: Fetal bovine serum.

Declarations

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Authors' contributions

YYW, XQZ, and XHP designed the study. YYW, YL, CT, QW, JH, JXW, and XQZ,carried out the experiments. YYW analyzed the data. WYY, XQZ, and XHP drafted and revised the paper. All authors read and approved the final manuscript for publication.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Experimental protocols were approved by the Experimental Animal Ethics Committee of the 920th Hospital of the PLA Joint Logistics Support Force.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Changes of PET/CT in thymus gland of macaques

	n	CT（HU）	SUVmax
Juvenile	5	56.00±6.24	1.70±0.36
Elderly-treatment	6	-14.67±10.07***	0.77±0.38*
90 days treatment	6	5.00±13.23	1.13±0.25
180 days treatment	6	48.33±17.39**	1.20±0.26

*P<0.05, ** P<0.01, *** P<0.001.

Figures

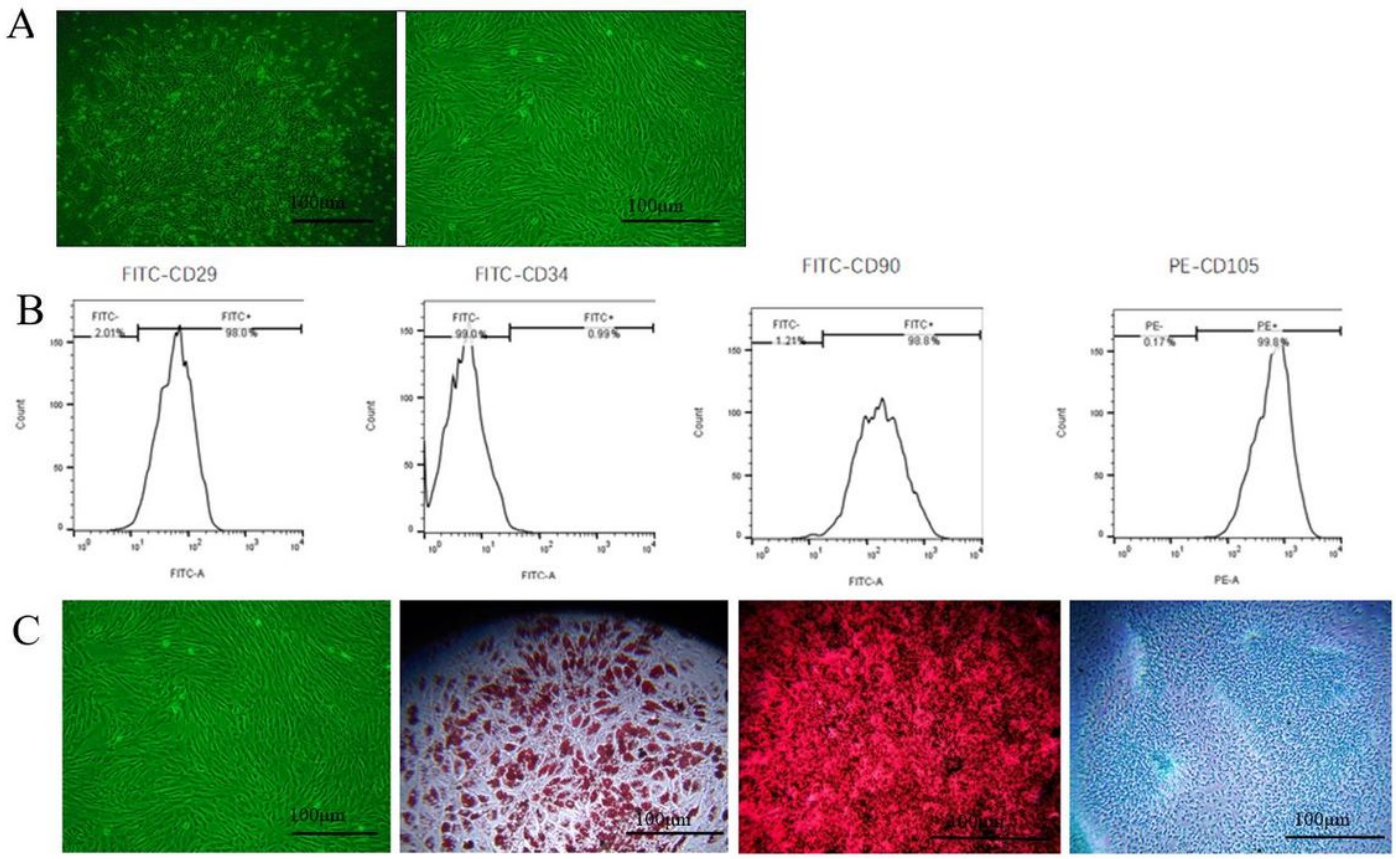

Figure 1

Preparation and identification of juvenile BMMSCs A The morphology of bone marrow mesenchymal stem cells adherent（100×） B Expression of juvenile macaque BMMSCs surface antigen C Identification of multiinduced differentiation of juvenile macaque BMMSCS（100×）

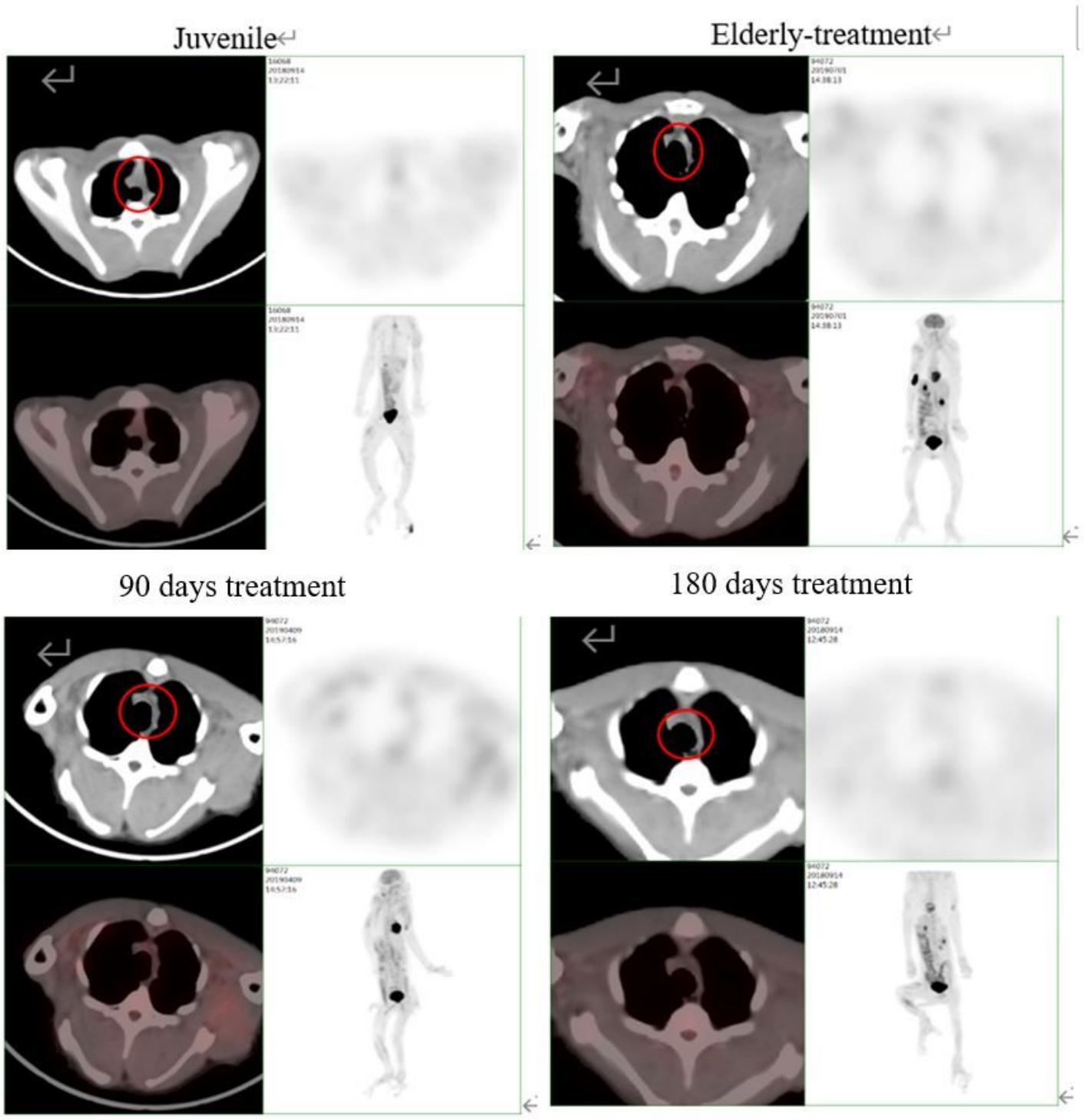

Figure 2

PET-CT changes of macaque thymus.

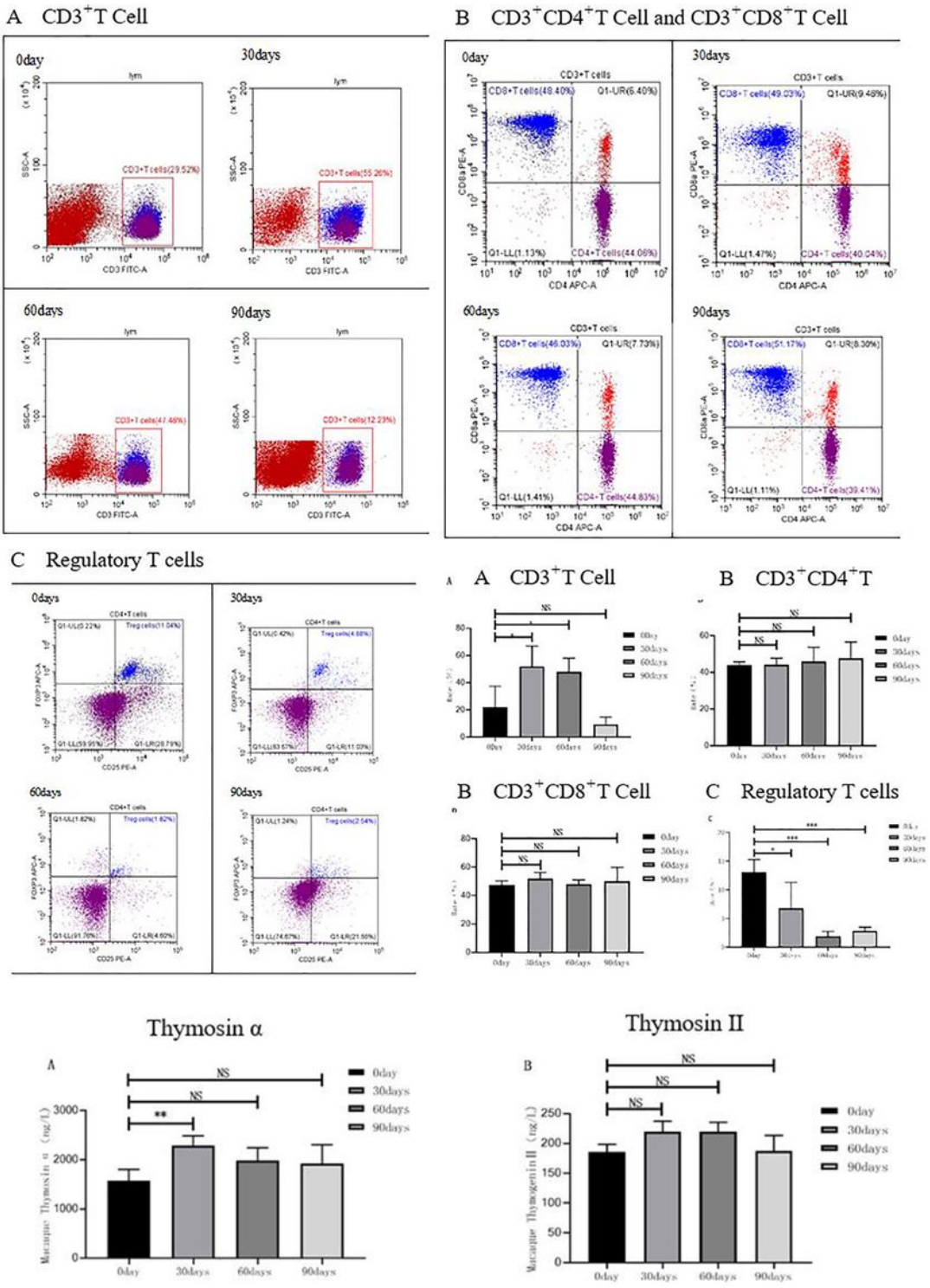

Figure 3

Changes in peripheral blood T cell and regulatory T cell output levels. a The level of CD3+ T cell output changes, * P＜0.05. b The level of CD3+ CD4＋T cell and CD3+ CD8＋T cell output changes, NS P＞0.05. c The level of regulatory T cell output changes, * P＜0.05，*** P＜0.0001. d Changes in thymosin α secretion levels, ** P＜0.01，NS P＞0.05. e Changes in thymopoietin Ⅱ secretion levels, NS P＞0.05.