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ORIGINAL RESEARCH

Investigation of the Underlying Mechanism of Sclerosteosis Expression in Muscle Tissue in Multiple Myeloma with Sarcopenia

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Objective: To explore the role of sclerosteosis (SOST) gene expression in the occurrence and development of multiple myeloma (MM) complicated with sarcopenia.

Methods: Analysis of the SOST expression in skeletal muscle tissue of patients with MM using high-throughput sequencing combined with transcriptomics; observation of morphological changes of the mouse C2C12 myoblasts co-cultured with SP2/0 myeloma cells in Transwell; observation of the SOST expression in the C2C12 myoblasts using the immunofluorescence labeling method; and assessment of the changes in exercise capacity of mice with MM using ethology; and the measurement of the SOST expression in muscles of mice using immunohistochemistry.

Results: The transcription level of the SOST gene in the muscle tissue was significantly higher in patients with MM and sarcopenia than in patients with MM without sarcopenia and elderly patients with sarcopenia; the area of C2C12 mouse myoblasts co-cultured with SP2/0 myeloma cells was 167,904 ± 8653.7 pix; this was significantly lower than the area of 402,994 ± 13,575.0 pix in the control group (CG); the fluorescence intensity of SOST in the cells of the experimental group (EG) was 159,389 ± 10,534 AU; this was significantly higher than the intensity of 26,338 ± 6059 AU in the CG; the differences in results of the coat-hanger test, the tail suspension test, the weight-bearing forced swimming test, and the grip strength test between the tumor-bearing mice in the EG and the CG were statistically significant; and the quantitative result of SOST expression in the muscle tissue of the EG mice was 11,515 ± 1573 pix; this was significantly higher than the result of 3399 ± 798.8 pix in the CG.

Conclusion: The SOST gene expression was significantly higher in muscle of mice in EG than in CG; and increased SOST gene expression might be a pathogenesis of MM complicated with sarcopenia.

Keywords: multiple myeloma, SOST, multiple myeloma mouse model, ethology, sarcopenia, myoblast differentiation

Introduction

Multiple myeloma (MM) is a malignant tumor characterized by the malignant proliferation of plasma cells. It accounts for approximately 10% of hematological tumors and 1% of all malignant tumors.[1] The incidence of MM varies greatly among countries and races, and there are no definite epidemiological data on the incidence of MM in China. With the research and application of various therapeutic methods in recent years, the efficacy of MM therapy has improved; however, the disease is still an incurable hematological malignancy. Currently, most research focuses on its treatment, and less attention is paid to the patients' quality of life and MM-related comorbidities. Moreover, there is still a lack of relevant research on MM complicated with sarcopenia.

Sarcopenia mainly refers to the loss of systemic skeletal muscle mass and the decrease in strength; these conditions are both related to age. Sarcopenia is commonly seen in elderly patients, patients with chronic diseases, and patients with

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cancer, which is not only related to age but also the prognosis of various tumors.[2–4] There have been previous researches on sarcopenia focused on osteoporosis, such as the study on the connection of sarcopenia and osteoporosis and the study on the extent of concomitance of sarcopenia and osteoporosis.[5,6] In recent years, a malignant tumor combined with sarcopenia has gradually attracted the attention of researchers, and it is believed that sarcopenia is related to the poor prognosis of various solid malignant tumors.[3,4,7] However, there are few studies related to MM and sarcopenia. According to the previous study of the present research group, the incidence of sarcopenia was much higher in patients with MM than in patients with age-related sarcopenia (the patients were in the same age group). The study also showed that sarcopenia might occur in the early disease stage in most patients with an unknown pathogenesis.

The pathogenesis of sarcopenia is complex and related to factors such as decreased exercise, weakened neuromuscular function, muscle cell apoptosis, nutrition, age-related hormonal changes, increased pro-inflammatory factors, and genetics. There are few studies on the molecular mechanism of sarcopenia. Some studies have shown that sarcopenia caused by senile osteoporosis may be related to the expression of sclerostin, which is a DAN family glycoprotein with a length of 190 amino acids that is well conserved across vertebrate species.[8,9] The sclerosteosis (SOST) gene, which encodes sclerostin, was identified as a novel gene by positional cloning as the sclerosteosis causative gene based on the discovery of three homozygous loss-of-function mutations in two unrelated sclerosteosis families and one isolated sclerosteosis patient.[10] Bone morphogenetic proteins (BMPs) and their interactions with steroid hormones in human osteoblasts have been demonstrated to affect SOST levels.[11] SOST has also been demonstrated to be a potent inhibitor of the Wnt signaling system, which it does by competitively binding to the Lrp5/6 cell surface receptors. The Wnt signaling pathway is critical for many physiological processes in cells and tissues.[12]

Therefore, the authors of this study speculate that the increase in the expression of muscle SOST might be one of the reasons for MM-induced sarcopenia. To verify this hypothesis, the following investigations are conducted: (1) the muscle tissues of the patients are collected to explore the changes of SOST expression in the muscle tissue of patients with MM and sarcopenia using high-throughput sequencing and bioinformatics analysis; (2) the changes in cell morphology and cell differentiation function are observed by co-culturing the myeloma SP2/0 cell line with the C2C12 myoblast cell line; (3) the changes in the SOST expression in the C2C12 myoblast cell line are observed using immunofluorescence labeling to establish the MM Bagg Albino (BALB)/c mouse model; and (4) the effects of MM on the exercise ability and muscle function of the BALB/c mice as well as the SOST expression in muscle tissue are observed using ethology detection methods and immunohistochemistry (IHC).

The present study intends to explore the related mechanism of MM-induced sarcopenia through the above-mentioned research contents as well as provide a basis for the in-depth investigation of the mechanism of MM's influence on muscles and the diagnosis and treatment of MM complicated with sarcopenia.

Materials and Methods

Experimental Objects

Tissue Sample

The paraffin-embedded skeletal muscle tissue samples were obtained from the Department of Orthopedics, Beijing Chaoyang Hospital, Capital Medical University. All patients signed informed consent and body tissue donation consent forms before sampling. All patients had no chemotherapy before operation. The general data of the samples are listed in Table 1. Mouse skeletal muscle tissue sections were all derived from MM mouse models.

Cell Strain

The C2C12 cells of the mouse myoblast cell line and SP2/0 cells of the MM rat cell line were both purchased from the American Type Culture Collection. (1) C2C12 myoblast cell line medium configuration method (DMEM medium +10% FBS serum +1% penystreptomycin mixture 100x): About 45mL DMEM medium was added with 5mlFBS serum and 500μL penicystreptomycin mixture (100x), respectively, and stored in a refrigerator at 4°C. (2) SP2/0 myeloma cell line medium configuration method (RPMI1640 medium +10%FBS serum +1% penystreptomycin mixture 100x): 45mL RPMI1640 medium was added with 5mL FBS serum solution and 500μL penicystreptomycin mixture (100x) and stored in refrigerator at 4°C. (3) C2C12 differentiation medium (DMEM medium +5% horse serum +1% penystreptomycin

Table 1 The Grouping of the Patients

Grouping	Number	Gender	Age (y)	Height (m)	Weight (kg)	Type of disease	Sarcopenia	RSMI (kg/m²)	BMI (kg/m²)
A	1	M	55	1.69	70.00	MM	N	7.54	24.50
	2	M	49	1.74	77.00	MM	N	7.71	25.80
B	1	M	52	1.73	58.00	MM	Y	6.53	19.38
	2	M	50	1.70	60.00	MM	Y	6.61	20.76
C	1	M	62	1.71	61.00	Lipoma	Y	6.90	20.86
	2	M	70	1.70	63.00	Spinal stenosis	Y	6.71	21.80

Note: (RSMI=The skeletal muscle mass of the four limbs(kg)/Height(m)², Sarcopenia: RSMI <7.26kg/m² in male; BMI= Weight (kg)/ Height (m)²).

mixture 100x): 2.5mL horse serum and 500μL penystreptomycin mixture (100x) were added into 47.5mL DMEM medium and stored in a refrigerator at 4°C.

Experimental Animals

The experimental animals were all BALB/c mice purchased from the Institute of Zoology, Chinese Academy of Sciences. They were fed by conventional feeding methods and randomly divided into two groups: the experimental group (EG) and the control group (CG) (n = 8 each). The mice in the EG were injected with SP2/0 myeloma cells intravenously and locally to establish the MM mouse model, and the model was verified by peripheral blood and bone marrow smears. The mice in the CG were injected with the same volume of RPMI1640 medium at the same site.

Experimental Methods

Sample Transcriptome Sequencing

The SOST expressions were analyzed using high-throughput sequencing of the skeletal muscle tissue from patients with MM; this was combined with transcriptomic analysis. Raw data quality control was conducted with the adoption of the fastp software. The sequence alignment analysis was conducted using TopHat2 and HISAT2 software.

Cell Experiments

Differentiation Induction Experiments of the Co-Cultured C2C12 Myoblast Cell Line

The C2C12 myoblast cell line co-cultured with SP2/0 cells was subjected to differentiation induction experiments, and the C2C12 myoblasts were induced to differentiate into muscle cells (Figure 1). Cells at logarithmic growth stage were taken for digestion by conventional methods and suspended in 1%FBS medium until the cell density was approximately (2–5)*10^5 /mL. Then, 400μL SP2/0 cell suspension was inoculated in the upper compartment of transwell chamber, and 400μL C2C12 cell suspension was inoculated in the lower compartment to 600μL medium containing 10% FBS (to avoid bubbles below the chamber). After cultured for 24h/48h, cells were taken out and put into a beaker. 800μL methanol/well was added to the 6-well plate. The upper chamber liquid was drained and fixed with the 4% paraformaldehyde solution at room temperature for 10–30min, and the staining was finished 20min after room temperature staining stability (crystal violet: the concentration of conventional working liquid is 0.1%). The decolorization was carried out by washing or soaking the chamber gently and repeatedly for at least 3 times with a large amount of flowing alkali water. The upper chamber of liquid was thoroughly drained and wiped with a clean fine cotton swab dipped in a little water. Five visual field cells were randomly observed under the microscope and photographed respectively. Using ImageJ graphics processing software, the image was transposed to 8-Bit format, and the gray value was measured to determine the number of cells. After co-culture, C2C12 myoblast cell lines were subjected to differentiation induction experiment. Differentiation induction experiment was performed on the co-cultured C2C12 myoblast cell lines. The original DMEM medium +10% FBS serum + 1% penicycin mixture 100x medium was replaced by DMEM medium +5% horse serum + 1% penicycin mixture 100x induction differentiation medium. C2C12 myoblasts were induced to differentiate into muscle cells. After being incubated in the incubator for 36h, the differentiation medium was abandoned and fixed with 4% paraformaldehyde cell fixating solution. Multiple fields were photographed under high magnification and images were converted into 8-Bit grayscale images using ImageJ image processing software. The gray value of the image cell area was measured to determine the muscle cell area and cell morphological changes.

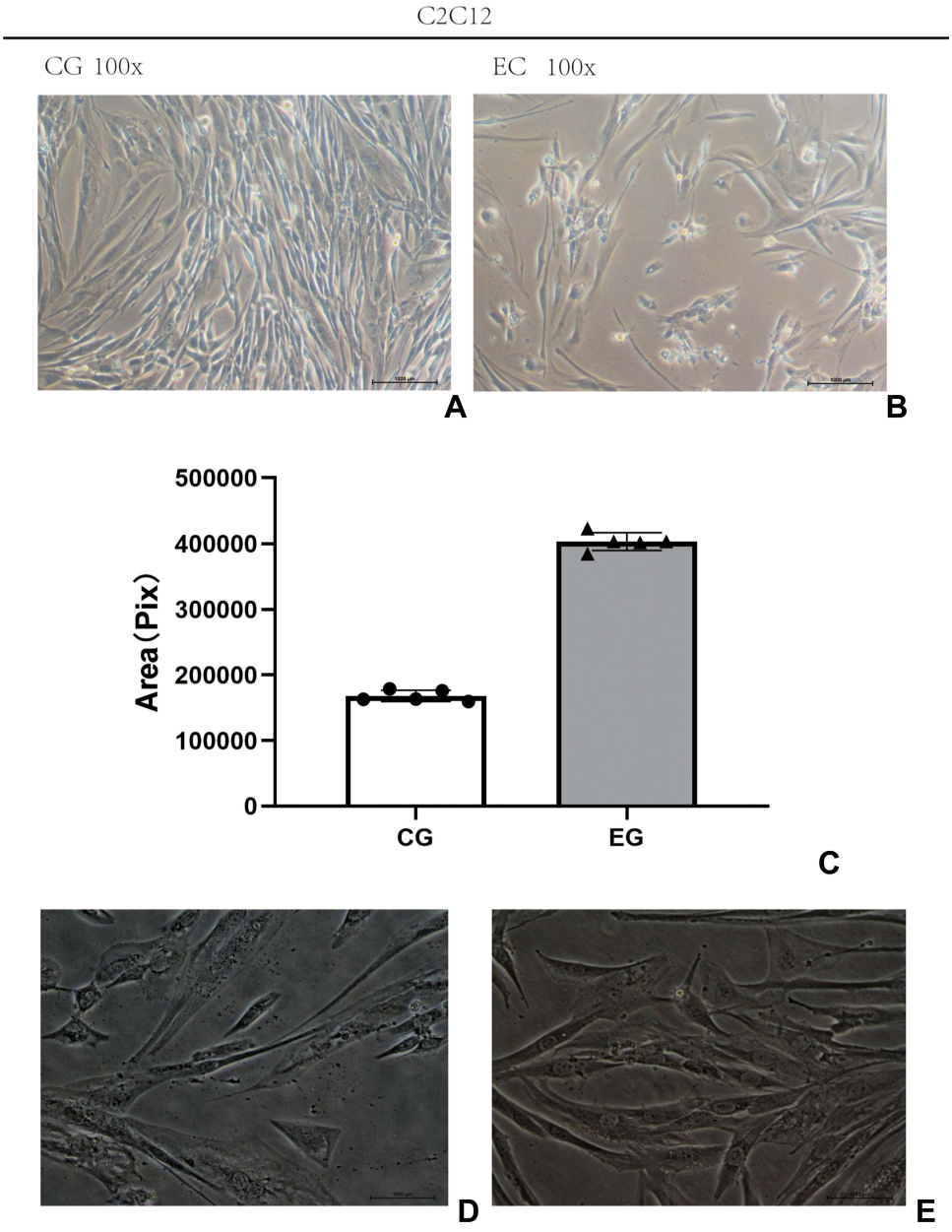

Figure 1 (**A** and **B**) The morphology of the C2C12 cell under a 100× microscope. (**C**) The mapping data of the cell area. (**D** and **E**) The morphology of the muscle cell fiber in the experimental group/control group under a 400× microscope.

Observation of the SOST Expression After Co-Culture of the C2C12 Myoblast Cell Line and SP2/0 Myeloma Cell Line Using Cell Immunofluorescence Staining

After co-culture and differentiation induction, the C2C12 myoblasts in the EG and the CG were fixed with 4% paraformaldehyde cell fixative for immunofluorescence staining (IFS). The cells in multiple fields of view were observed under a fluorescence microscope for screenshots, and the ImageJ graphics processing software was used to segment the cells in the RGB color channels. The DAPI fluorescence images were observed under the blue channel and the SOST fluorescence images were observed under the green channel; the images were merged to generate MERGE images. Transwell co-cultured SP2/0 myeloma myoblasts and C2C12 myoblasts were located in the superior compartment and inferior compartment, respectively. All co-cultures were Transwell co-cultures.

Animal Experiments

The BALB/c mice were injected with SP2/0 myeloma cells into the tibia and tail veins to create a MM mouse model, and the model was verified by bone marrow and blood smears. The exercise ability and muscle strength of the mice in the CG and EG were tested using the coat-hanger test, the tail suspension test, the mouse grip strength test, and the weight-bearing forced swimming test. The experiment was conducted 4 weeks after the successful establishment of the MM mouse model.

Weight-Bearing Swimming Experiment

The determination of the initial time of swimming after loading mice into the water: the tail of each group of mice were hung with 10% of the weight of the lead wire, put into a water depth of about 40 cm, high water temperature of about 35°C pool swimming, starting from the water timing, when the mice have completely sunk into the pool water and cannot float back to the surface of the water, that was, the loading water termination timing.

Hanger Experiment

The test mice in the above groups were respectively suspended to a hanger-like instrument with the width, height and length of about 40 cm from the experimental platform. At the beginning, the mice were helped to grasp the middle of the horizontal bar with their front PAWS. At the same time, time was started and the time after the mouse PAWS fell down vertically from the horizontal bar was observed and recorded.

Tail Suspension Experiment

The mouse tail was fixed on the plate, and the head end was suspended downward, and the duration of the mouse's first struggle was recorded until the mouse was stationary and swayed for more than 3 seconds without any obvious struggle. After the experiment of each group of mice, the above experiment was repeated with a rest of half an hour, and the experiment was repeated for 3 times in each group. The final average value was taken as the result.

Grip Strength Test

The mouse was lift by holding the tail, and put on the mouse grip strength tester. When the mouse forelimbs gripped firmly, the mouse tail was pulled backward slowly and the strength was gradually increased until the mouse forelimbs released the grip rod. The final grip strength data of the mouse on the tester was recorded. This process was repeated for 3 times, and the average value of the data was used as the final result.

Statistical Methods

Non parametric tests were used in studying the differences of the SOST gene expression in the muscle between patients with MM with sarcopenia and patients with MM without sarcopenia and between patients with MM with sarcopenia and elderly patients with sarcopenia; and the differences of the SOST expression and area between the mouse C2C12 myoblast cell and the C2C12 myoblast cell after co-culture with SP2/0 myeloma cell; and the differences of the exercise ability and the SOST expression between the MM tumor-bearing mice and the control group mice.

Results

Transcriptomic Studies in the Muscle Tissue of Patients with MM

Differential Expression in the Muscle Tissue Samples of Patients with MM and Sarcopenia and Patients with MM Without Sarcopenia

Transcriptomic analysis was conducted on the muscle samples from patients with MM and sarcopenia, patients with MM without sarcopenia, and elderly patients with sarcopenia. The sample sequencing was performed by Shanghai Majorbio Co., Ltd. First, random sequence extraction and detection of the RNA sequences in the sample library and analysis of the structure of the transcriptomic library constructed after hybridization and pairing with the parents were conducted, and it was ensured that the gene expression and quality detection in the sample sequences of the sequenced samples were all qualified or correct; then, the samples were loaded on the machine for sequencing. Through the analysis of the bioinformatics characteristics of the sequenced sample sequences, the following results were obtained: (1) a total of 32,835 expressed genes and 155,060 transcripts were detected in the sequence analysis of the six samples, in which a total of 2891 differentially expressed genes

(including 1807 up-regulated genes and 1084 down-regulated genes) were detected in the muscle tissue of patients with MM combined with severe sarcopenia and patients with MM without sarcopenia; and (2) a total of 4302 differentially expressed genes (including 1454 up-regulated genes and 2848 down-regulated genes) were detected in the muscle tissue of patients with MM and sarcopenia and elderly patients with severe sarcopenia (Figure 2A–C).

Through a principal component analysis, it was revealed that the differences in the gene expression among the three groups were statistically significant ($p < 0.05$), while the differences within the groups were not statistically significant ($p > 0.05$) (Figure 2D). The gene expressions of the samples in each group were equal (Figure 2E); all samples were qualified by quality control.

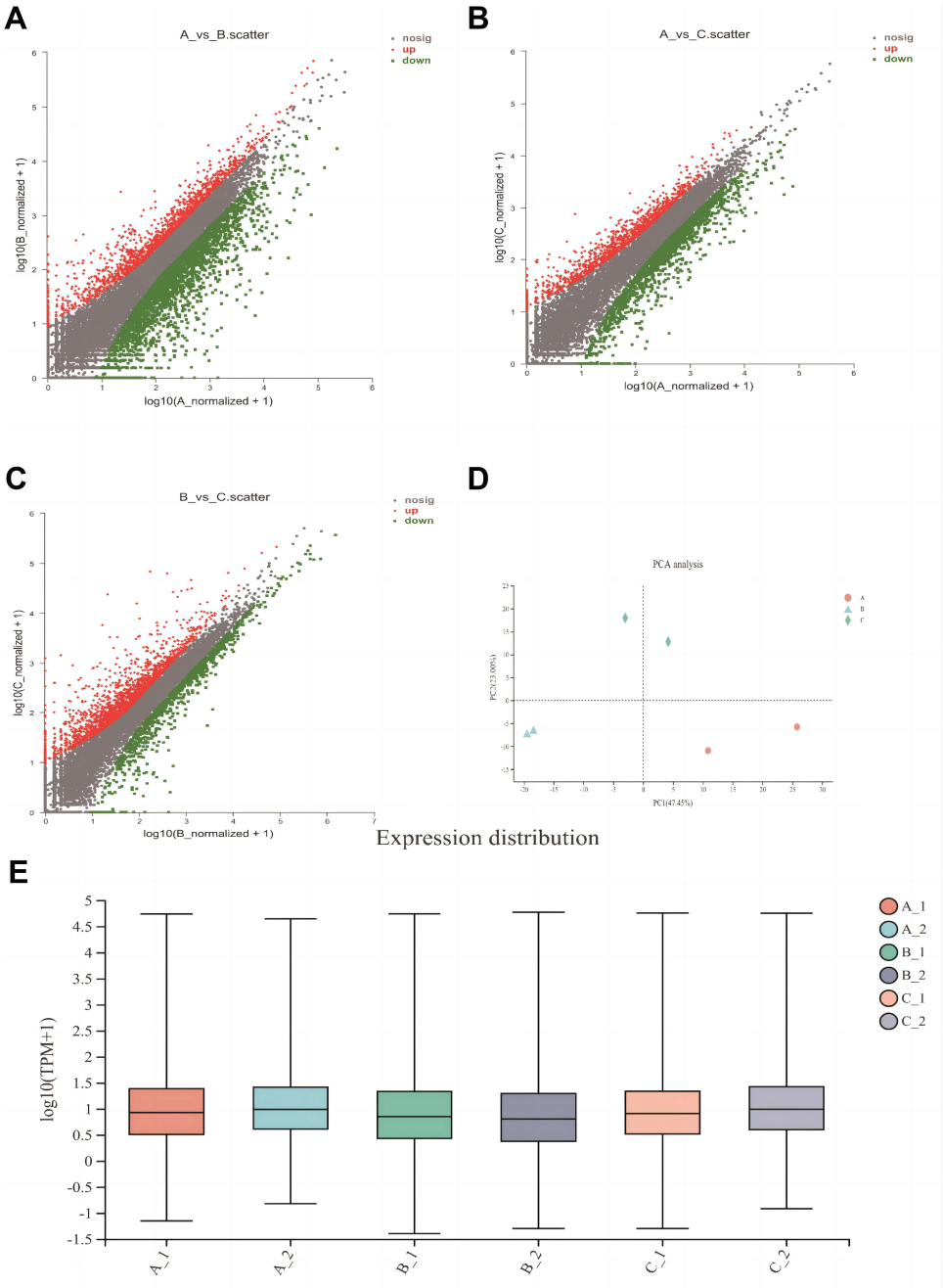

Figure 2 (**A**) The scatter plot of the differential expression in the muscle tissue between patients with multiple myeloma (MM) without sarcopenia and patients with MM and sarcopenia. (**B**) The scatter plot of differential expression in the muscle tissue between patients with MM without sarcopenia and elderly patients with sarcopenia. (**C**) The scatter plot of differential expression in the muscle tissue between patients with MM and sarcopenia and elderly patients with sarcopenia. (**D**) The scatter plot of the differences among the three groups of samples and the differences within the groups. (**E**) The distribution of expressions among different samples.

Differential Expression of the SOST Gene in the Muscle Tissue of Patients with MM and Sarcopenia

After summarizing the differentially expressed genes, gene ontology enrichment analysis was conducted with the adoption of the cluster package. It was found for the first time that SOST gene expression was significantly reduced in the muscle tissue of patients with MM without sarcopenia and significantly increased in the muscle tissue of patients with MM and sarcopenia. The results of the Kyoto Encyclopedia of Genes and Genomes enrichment analysis showed that some differential genes could be enriched in the Wnt signaling pathway; among these genes, the differences in the SOST expression were particularly obvious (shown in red in Figures 3A–C). The SOST expression increased slightly in the subjects with sarcopenia without MM and increased significantly in the muscle tissue of patients with MM and sarcopenia; in addition, the SOST gene was not transcribed in the muscle tissue of patients with MM without sarcopenia (Figure 3D).

Cell Experiments

To explore the influences of myeloma cells on the muscle tissue of tumor-bearing mice, the effects of the mouse SP2/0 myeloma cell line on the expression, proliferation, and differentiation ability of the SOST gene of the mouse C2C12 myoblast cell line were observed using Transwell co-culture experiments.

Observation of the Effect of SP2/0 Myeloma Cells on the Proliferation and Differentiation of the C2C12 Myoblasts Using the Transwell Co-Culture Experiment

The results of the Transwell experiment are demonstrated in Table 2 and Figure 4: the number of proliferated cells in the C2C12 myoblast cell line in the EG at 24, 48, and 96 h were significantly lower than the corresponding numbers of the C2C12 myoblast cell line in the CG; the differences were statistically significant ($p < 0.001$). Meanwhile, it was suggested that the slope of the cell proliferation curve decreased; however, the differences between the two groups were not statistically significant ($p > 0.05$). Through microscope observation after the cells in the EG were co-cultured for 96 h, it could be observed in the medium for clear and translucent cell debris that the SP2/0 myeloma cell line significantly inhibited the growth and cell activity of the C2C12 myoblast cell line.

Differentiation Induction Experiment of the Co-Cultured C2C12 Myoblast Cell Line

The results showed that the area of the newly formed muscle fibers in the EG was significantly reduced after differentiation when compared with the CG ($p < 0.05$) (Table 3). Meanwhile, the cells lost their parallel and regular morphology, and the muscle fibers appeared to be curled and deformed (Figures 1A–C). Under high magnification (400×), it could be seen that the muscle cell fibers were thinned (Figures 1D and E).

Observation of the SOST Expression After Co-Culture of the C2C12 Myoblast Cell Line with the SP2/0 Myeloma Cell Line Using IFS

The results are illustrated in Figure 5 and Table 4: the results suggested that the SOST gene expression in the differentiated C2C12 cells was significantly increased in the EG when compared with the CG, with the expression site located in the cytoplasm; the difference was statistically significant ($p < 0.01$).

The above experiments showed that in the myoblasts affected by MM cells, the SOST gene expression was increased in the cytoplasm; meanwhile, the growth and cell activity of the myoblasts were tied together with the morphological changes of cell differentiation. Thus, MM cells might affect the proliferation and differentiation of myoblasts.

Animal Experiments

Construction of the MM Mouse Model

A large number of myeloma cells were observed in the peripheral blood and bone marrow smears of the mice with MM; this proved that the MM mouse model was constructed successfully. The results are shown in Figure 6.

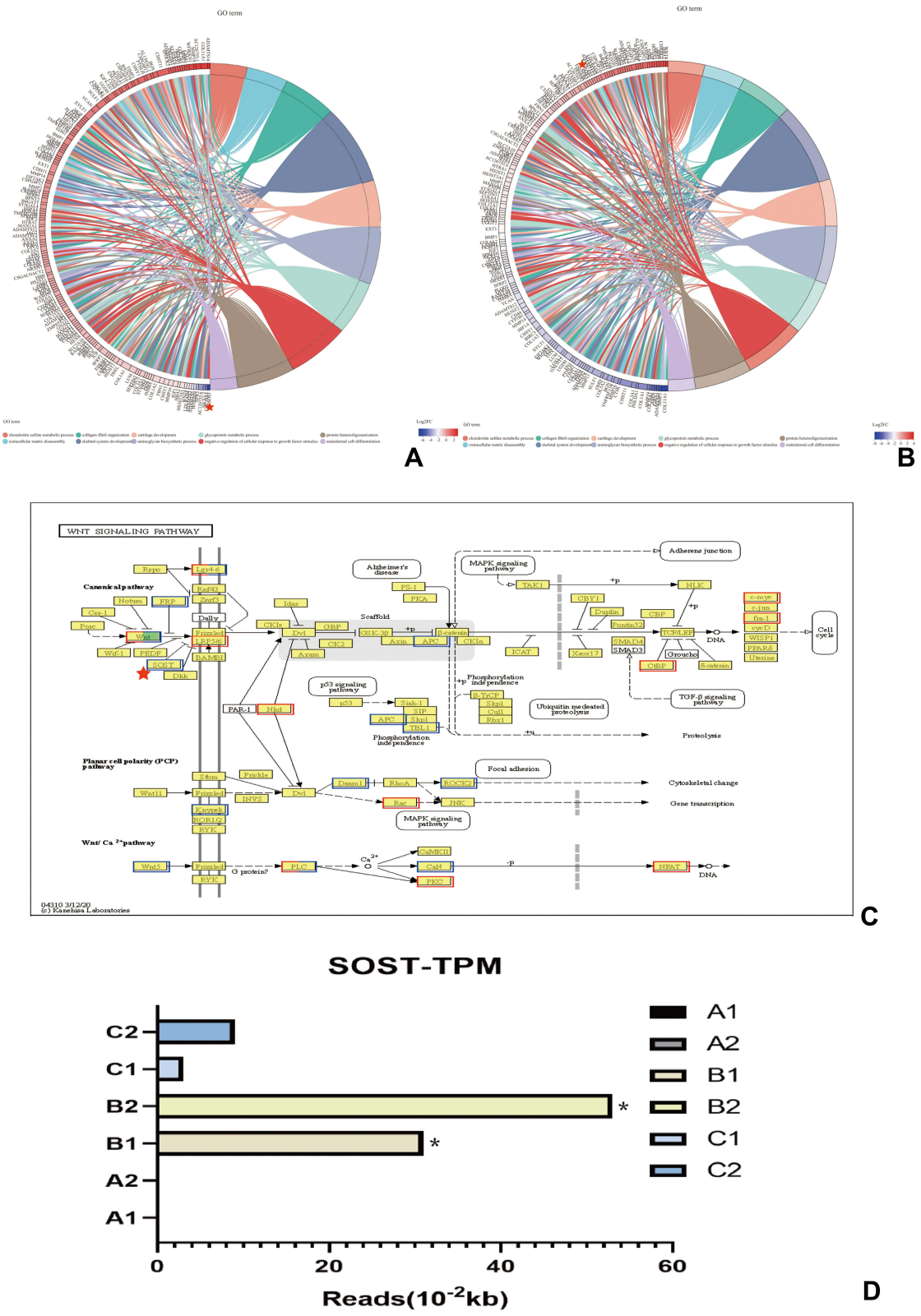

Figure 3 Kyoto Encyclopedia of Genes and Genomes enrichment analysis of the sclerosteosis (SOST) gene expression in (**A**) patients with MM and sarcopenia, and (**B**) patients with sarcopenia without MM. (**C**) The Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of the differentially expressed genes in patients with MM without sarcopenia and patients with MM and sarcopenia showed that the SOST genes and partial Wnt signaling pathway genes. (**D**) Comparison of the expression levels of SOST among the three groups.

Table 2 The Measurement Data of the Cell Culture (Unit: Pixel)

Group	Label	Area	%Area
EG	24h	19,233±1053.9	1.57
	48h	56,350±2386	4.59
	96h	82,743±3782	9.94
CG	24h	91,162±4835	7.42
	48h	91,162±4835	12.08
	96h	212,856±7953.9	17.32

The Exercise Ability and Muscle Strength Was Significantly Decreased in the Tumor-Bearing Mice with MM in the EG Compared with the Mice in the CG

The coat-hanger test, tail suspension test, mouse grip strength test, and weight-bearing forced swimming test were adopted to evaluate the exercise ability and muscle strength of mice in the CG and EG.

Coat-Hanger Test

The experimental results showed that the duration of the upper limb suspension of the tumor-bearing mice with MM was 1.38 ± 1.30 s; this was significantly shorter than the 13.75 ± 4.10 s in the CG mice ($p < 0.01$). The results are shown in Figure 7A.

Tail Suspension Test

The EG results showed that the struggling time of the tumor-bearing mice with MM was 23.50 ± 7.33 s; this was significantly lower than the 65.88 ± 10.36 s in the CG mice ($p < 0.01$). The results are shown in Figure 7B.

Forced Swimming Test

The swimming endurance time of the tumor-bearing mice in the EG was 45.88 ± 8.81 s; this was significantly lower than the 85.25 ± 20.60 s in the CG mice ($p < 0.01$). The results are illustrated in Figure 7C.

Grip Strength Test

It was found that the grip strength of tumor-bearing mice in the EG was 29.23 ± 4.98 N^{-2}; this was significantly lower than the 59.38 ± 12.60 N^{-2} in the CG mice ($p < 0.01$). The results are shown in Figure 7D.

Increased SOST Expression in the Muscle Tissue of Tumor-Bearing Mice Verified by IHC

The mice in the EG and CG were sacrificed eight weeks after tumor bearing. Approximately 1.5 cm of the gastrocnemius tissue on the ipsilateral hindlimb of the injection site was taken. The IHC analysis showed that the SOST expression of the muscle tissue sample sections was significantly higher in the EG than in the CG; the difference was statistically significant ($p < 0.01$). The results are demonstrated in Figure 8 and Table 5.

Discussion

Pathogenesis of MM with Sarcopenia

In recent years, malignant tumors complicated with sarcopenia have gradually attracted the attention of researchers.[3,4,7] However, studies on the relationship between MM and sarcopenia are lacking. The research group found that the prevalence of sarcopenia in MM patients was 48% based on previous clinical data; this is significantly higher than the findings of Taiwanese researchers on the prevalence of sarcopenia in the general population.[4] Korean researchers suggested that the prevalence of sarcopenia in elderly males and females was 9.3% and 4.1%, respectively,[13] while the prevalence of sarcopenia in elderly Japanese males and females was 9.6% and 7.7%, respectively.[12] This research group found that the prevalence of sarcopenia in males and females was 32.6% and 71.26%, respectively (Ren Jie et al,

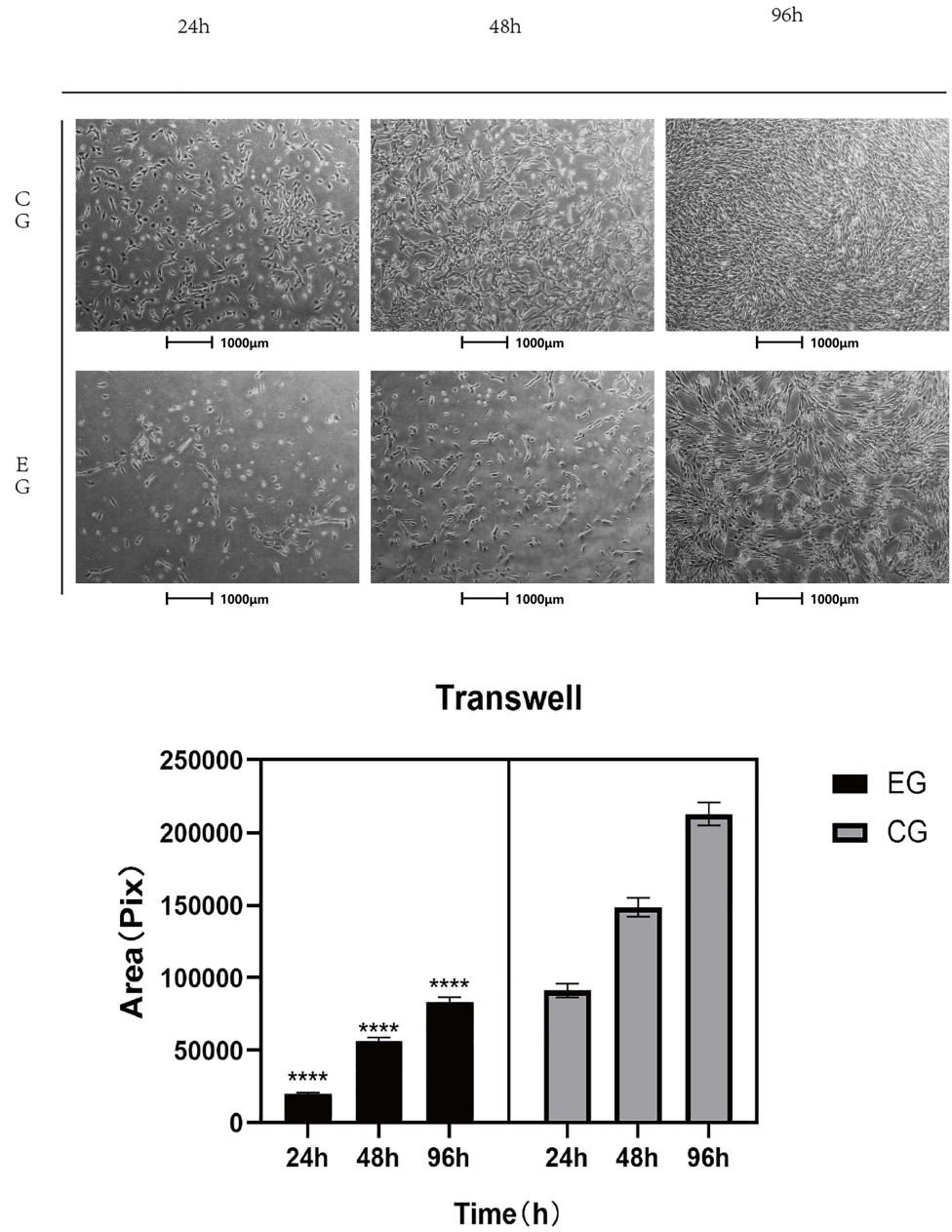

Figure 4 Results of the Transwell experiment. Control group: The growth of cells in the control group after 24, 48, and 96 h. Experimental group: The growth of cells in the experimental group after 24, 48, and 96 h. Area: The plot of cell picture measurement data.

Chinese Orthopedic Journal for publication). Therefore, in this study, the pathogenesis of sarcopenia in patients with MM was explored from the aspects of bioinformatics analysis and cell experiments to better diagnose and treat patients with MM and sarcopenia.

Table 3 The Mapping Data of the Cell Area

Label	Area	%Area
EG	167904±8653.7	12.82
CG	402994±13,575	36.61

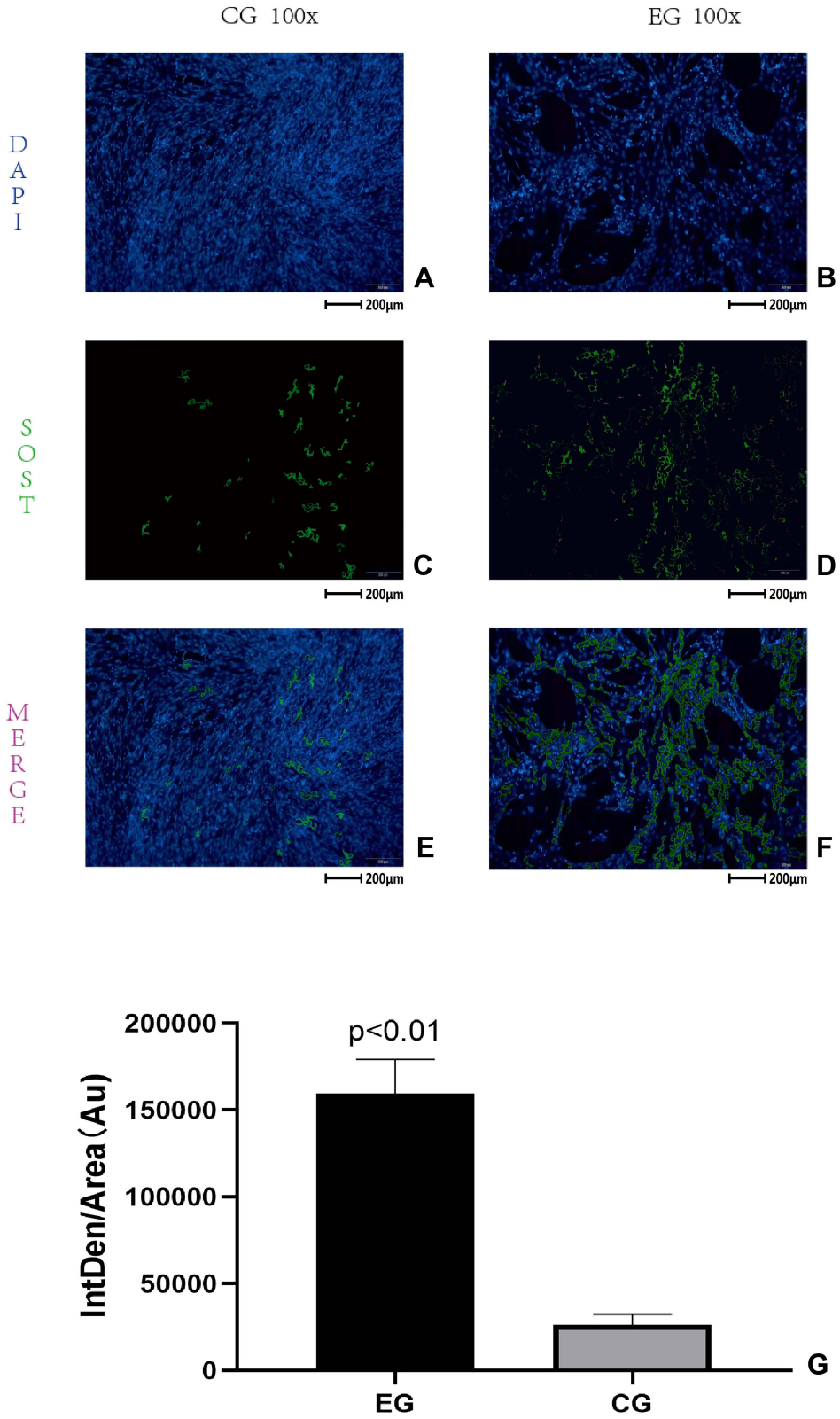

Figure 5 (**A** and **B**) The DAPI imaging of cell counterstaining. (**C** and **D**) The green fluorescence imaging of the sclerosteosis (SOST) gene in cells. (**E** and **F**) The MERGE imaging of cell immunofluorescence. (**G**) The plot of the SOST expression measurement.

Table 4 The Detection Data of the Cell Fluorescence Measurement

Label	Area	%Area
EG(green)	159,389±10,534	6.91
CG(green)	26,338±6059	1.14

Regarding the mechanism of sarcopenia, previous studies focused on the following aspects: the hepatocyte growth factor signaling pathway, oxidative stress, the reactive oxygen species and oxidative stress pathway, the inflammatory mechanism, and neurological and brain-derived neurotrophic factors. Currently, there is no clear conclusion regarding the mechanism, indicating that sarcopenia may be controlled and caused by a variety of factors.[14]

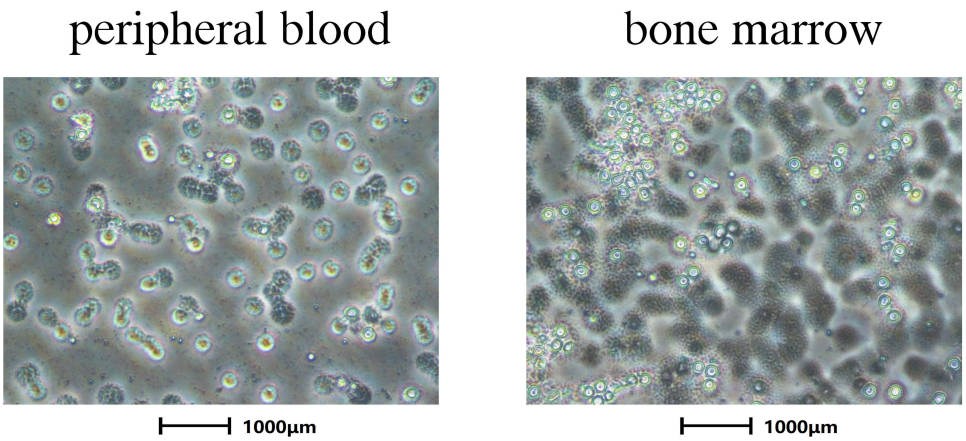

Figure 6 Myeloma cells in the peripheral blood and bone marrow of mice with MM.

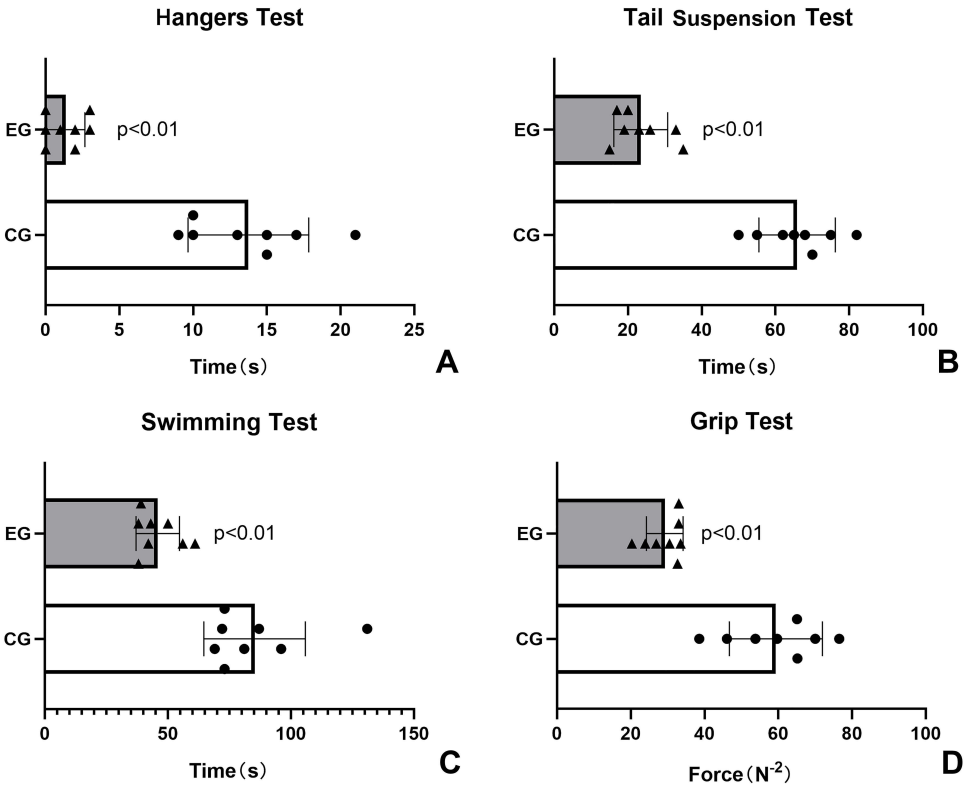

Figure 7 (**A**) The coat-hanger test. (**B**) The tail suspension test. (**C**) The forced swimming test. (**D**) The grip strength test.
Abbreviations: CG, the control group; EG, the experimental group.

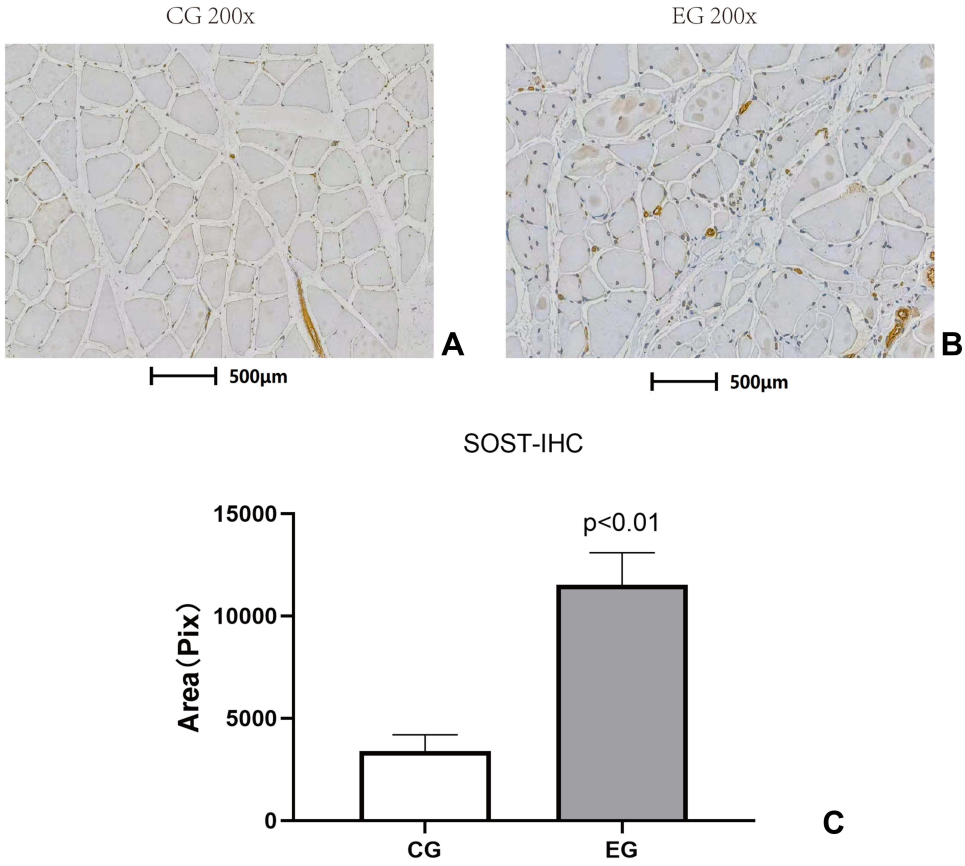

Figure 8 The SOST expression in the muscle tissue of mice with MM. The IHC analysis showed that the SOST expression of the muscle tissue sample sections was significantly lower in the CG (**A**) than in the EG (**B**); the difference was statistically significant ($p < 0.01$) (**C**).
Abbreviations: CG, the control group; EG, the experimental group.

With the adoption of high-throughput sequencing technology, the authors of the present study conducted transcriptomic analyses of the muscle tissue samples from patients with MM and sarcopenia, patients with MM without sarcopenia, and elderly patients with sarcopenia. The results showed that the SOST expression in the muscle tissue was significantly higher in patients with MM and sarcopenia than in elderly patients with sarcopenia; this suggested that the higher incidence of sarcopenia in patients with MM than in people without MM might be correlated with the abnormal increase in the SOST expression in the muscle.

Correlation Between SOST and MM Complicated with Sarcopenia

Sclerosteosis mainly expresses sclerostin in the human body; it is a typical inhibitor of the Wnt signaling pathway that is considered to be an important regulator of bone homeostasis. A large amount of research highlights the potential therapeutic significance of sclerostin under many pathological conditions. Clinical studies have also found that SOST gene expression is correlated with more than just osteoporosis.[15] A bone morphogenetic protein (BMP) antagonist, the

Table 5 The Detection Data of Immunohistochemistry Assay (Unit: Pixel)

Label	Area	%Area
EG	11515±1573	1.65
CG	3399±798.8	0.49

sclerostin protein is produced by the SOST gene and may prevent BMP from binding to its receptor.[16] Sclerostin suppresses the Wnt signaling pathway by binding to LRP5/6. This prevents mesenchymal stem cells from differentiating into osteoblasts while causing osteoblast death. Myoblasts, which originate from mesenchymal stem cells and can interconvert with osteoblasts and chondrocytes under specific circumstances,[17] are muscle satellite cells. Skeletal muscle satellite cell growth and differentiation are essential for skeletal muscle regeneration. Maintaining the satellite cell pool and its regeneration capabilities depend on skeletal muscle satellite cells exhibiting the correct balance of quiescence, proliferation, and differentiation. Therefore, the authors of this study speculated that the reason why patients with MM are more prone to sarcopenia might be correlated with the imbalance of muscle proliferation and differentiation ability caused by high SOST expression in the myoblasts in muscle tissue. The experimental results of this study further confirmed this inference.

Clinical Significance of in vitro and Animal Experiments in MM Complicated with Sarcopenia

To simulate the physical state of patients with MM, an MM tumor-bearing mouse model was constructed based on the existing literature.[14] During the progress of the disease, the exercise ability and muscle strength were tested through ethological experiments to confirm that patients with MM were more prone to developing the symptoms of reduced exercise capacity and muscle strength. Meanwhile, the muscle samples were stained using IHC, and it was found that the SOST expression in the muscle tissue of the tumor-bearing mice was significantly increased. The results of the animal experiments were consistent with the clinical observations and the sequencing results of SOST in the above-mentioned muscle tissue, further proving that high SOST gene expression might result in an imbalance of proliferation and differentiation of myoblasts as well as the inhibition of the transformation of myoblasts to muscle.

A Transwell co-culture experiment of the MM cell line and myoblast cell line was designed and conducted. It was found in this study that the myoblast co-cultured with the MM cell line showed a significantly reduced proliferation and differentiation ability; at the same time, the differentiated muscle fibers lost their regular morphological features. Meanwhile, the myoblast cell lines in the CG showed normal biological characteristics of myoblasts in the experiments of proliferation and differentiation induction, indicating that the MM cell line indeed affected the proliferation and differentiation of the myoblast cell line.

To further confirm the above findings, the cellular IFS detection of SOST was conducted in the co-cultured and induced differentiated myoblasts, and the normally differentiated myoblasts were taken as the control. The result revealed that the SOST expression in the cytoplasm significantly increased in the myoblasts co-cultured with the MM cell line, while only a small amount was expressed in the CG. Furthermore, the SOST expression was negatively correlated with the number and area of muscle cells. Therefore, the authors of this study believe that the abnormal expression of SOST in the muscle tissue of patients with MM will inhibit the proliferation and differentiation of myoblasts in the muscle tissue, thereby reducing the activity of muscle regeneration; this could theoretically explain the occurrence of sarcopenia at an early disease stage in patients with MM. Certain articles report that the transformation and activation of fibroblasts into myoblasts is closely correlated with BMP and that SOST, as a known BMP, might have an inhibitory effect on this transformation and activation. Furthermore, certain pieces of literature report that an increased SOST expression may inhibit the activation of the Wnt pathway in chondroblasts and osteoblasts, thus inducing apoptosis.[18,19] The results of the present study proved the above points of view from another perspective while indicating that the same phenomenon might exist in the myoblast pool of muscle tissue in patients with MM; that is, an increase in SOST expression might result in the inhibition of BMP and the transformation of fibroblasts to myoblasts. This requires further investigation.

In conclusion, SOST is expected to become a new therapeutic target for relieving early sarcopenia in patients with MM, and it is of great significance for improving the prognosis of patients with MM. This could lay a foundation for further study on the cell signaling pathway of the abnormal SOST expression in myoblasts caused by MM.

Correlation Between MM Complicated with Sarcopenia and Osteoimmunology

In order to establish a clear cross-talk between the immune system and the bone niche in both physiological and pathological activities, including cancer, osteoimmunology was first examined in 2000.[20] A hematologic cancer known as multiple myeloma (MM) is caused by the clonal proliferation of malignant plasma cells in the bone marrow. Adverse SREs are frequently linked to this condition. The ability of the bone lesions in MM to accelerate the processes of bone resorption depends on their lytic nature. The displacement of the RANKL/OPG ratio to osteoclastogenesis is the main mechanism.[21] A putative locoregional (bone) and systemic cancer control could be explained by a two-way process between cancer cells and bone niche components. We might surmise that the immune system functions as a "bond", a link between cancer cells and bone niche cells. A thorough understanding of this complicated equilibrium can serve as a possible therapeutic target to reduce systemic cancer pathology, including MM with sarcopenia,[22] in addition to bone metastases.

Conclusion

1. The SOST gene expression in the muscle tissue samples was significantly higher in patients with MM and sarcopenia than in patients with MM without sarcopenia and elderly patients with sarcopenia.
2. The SP2/0 MM cell line might induce the increase of SOST expression in the cytoplasm of the C2C12 myoblasts and inhibit the proliferation and differentiation of the C2C12 myoblasts, suggesting that the abnormal expression of SOST in the muscle tissue of patients with MM will inhibit the proliferation and differentiation of myoblasts in the muscle tissue.
3. The muscle exercise activity was significantly weakened in tumor-bearing mice with MM when compared with the control mice, and the SOST gene expression in the muscle tissue of the tumor-bearing mice was significantly increased when compared with the control mice, suggesting that the abnormal expression of SOST may reduce the activity of muscle regeneration.
4. An increased expression of the SOST gene might be a pathogenesis of MM complicated with sarcopenia.

Limitations and Shortcomings

1. All muscle specimens were obtained from males, and there may be a gender bias.
2. Most patients with MM received other treatments, such as chemotherapy, and the influences of such treatments and other factors cannot be excluded.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of Beijing Chao-yang Hospital. This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants. All animal experiments were conducted according to the guidelines established by the National Institutes of Health (http://grants1.nih.gov/grants/olaw/) and approved by Animal Use Committee of National Drug Clinical Trial Institution, Beijing Chao-yang Hospital.

Disclosure

The authors declare that they have no competing interests in this work.

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