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Research Article

Adipose mesenchymal stem cells overexpressing chemokine (CXCL6) showed strong angiogenesis and anti-inflammatory ability in the treatment of pulmonary hypertension

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Received Date: January 09, 2024; **Accepted Date:** January 16, 2024; **Published Date:** January 23, 2024

Citation: Wen Wen, Liu Hao, Ba Ming Chuan, Li Li Jun, Guo Lin, et al, (2024), Adipose mesenchymal stem cells overexpressing chemokine (CXCL6) showed strong angiogenesis and anti-inflammatory ability in the treatment of pulmonary hypertension, *J. Biomedical Research and Clinical Reviews*. 9(1); DOI:**10.31579/2692-9406/177**

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Abstract

Objective: Pulmonary arterial hypertension (PAH) is a disease characterized by persistent pulmonary vascular remodeling, and its pathogenesis remains unclear. The traditional treatment of pulmonary hypertension is not good. Many researchs have been done on stem cell treatment. We have found that Adipose mesenchymal stem cells (ADMS) can be used in the treatment of pulmonary hypertension, and stem cells can greatly enhance the proliferation of stem cells if transfected with certain chemokines. CXCL6 is such a chemokine, CXCL6 is also called GCP2, but the efficacy of ADMS-transfected CXCL6 for PAH is still lacking.

Methods: Pre-Clinical Research Report. We designed cell and animal studies in which CXCL6 was transfected with ADMS. The differences of cell migration and vascular tubular ability between ADMS transfected with GCP gene and ADMS alone were detected, and the therapeutic effect of ADMS transfected with GCP gene was verified in animal models, so as to determine the specific effects of ADMS transfected with single gene at the molecular, cellular and global levels.

Results: The experimental results confirmed that GCP2 transfected with ADMS had stronger angiogenesis and anti-inflammatory ability in the treatment of pulmonary hypertension diseases compared with ADMS.

Conclusion: This proved that ADMS -transfected chemokines could further enhance cell proliferation and anti-inflammatory ability.

Key words: adipose mesenchymal stem cells; granulogyte chemotactic protein-2; angiogenic; anti-inflammation; model of pulmonary arterial hypertension

Abbreviations:

PAH: Pulmonary arterial hypertension,

ADMS: Adipose mesenchymal stem cells,

CXCL6: Chemokine 6,

MCT: monocrotalin,

1.Introduction

Pulmonary arterial hypertension (PAH) is a disease characterized by progressive pulmonary vascular remodeling, resulting in right-sided heart failure and premature death [1]. Current available therapies for PAH, including the endothelin-1, prostacyclin, and nitric oxide pathways use of targeted medicine therapy. However, PAH patients treated with targeted can expect a mean survival of 7 years [2]. Therefore technological development of more effective therapy agents for the treatment of PAH[2]. Cell therapy may offer a novel therapeutic approach to PAH. Adipose mesenchymal stem cells (ADMS) are one of the most well-characterized stem cells that can be multimodal ability not only in terms of differentiation but also in their secrete consisting of various cytokines,

chemokines, and growth factors related to multiple cellular function including angiogenesis, anti-apoptosis, and antiinflammation [3,4].

However, recent studies have identified some impediments, including a low survival rater for transplanted stem cells demonstrated in an PAH model[4] . It has been reported that chemotactic cytokines play a pivotal role in angiogenesis, immunity, and tumorigenesis [4,5]. Recent reports demonstrate that the overexpression of Chemokine 6, (CXCL6) results in enhanced angiogenesis [1,6]. CXCL6 is also called GCP2. In particular, overexpressed GCP2 in tumour cell allografts results in enhanced angiogenesis [7]. Previously, our group demonstrated the role of GCP2 and the interactions between GCP2, vascular endothelial growth factor in a hindlimb ischaemia model [8].

Recent studies found that GCP2 as a specific vascular endothelial growth factor has the functions of stimulating endothelial cell proliferation and migration, and inhibiting cells apoptosis and Revascularization [9], however, its not clear whether GCP 2 was able to attenuate the inflammatory response in PAH.

The purpose of this study was to investigate changes of pulmonary hemodynamis, inflammatory response, and prevention pulmonary arterial remodelling in monocrotali(MCT）induced PAH after GCP2 genen transfer of ADMS.

2.Materials and methods

2.1 Main reagents and instruments

Main reagents: DMEM (BI), α-MEM medium (Procell), fetal bovine serum (Gibco), antibodies CD29-APC and CD45-PE (Ebioscience), GCP2 antibody (Wuhan Sanying), Monocrotaline (Ald), ELISA kit (Yitlaier). Main instruments: real-time fluorescence quantitative PCR (ABI), micro-spectrophotometer (Hangzhou Aoshengyi Doris Instrument System Co., Ltd.) [8].

2.2 Cultivation of rat adipose mesenchymal stem cells (ADMS)

Primary adipose mesenchymal stem cells were purchased from Shanghai Cybertron [8]. Remove the cells from liquid nitrogen and quickly dissolve them in a 37°C water bath. After dissolution, transfer the cells to a centrifuge tube containing 5ml culture medium, centrifuge and discard the supernatant; resuspend in complete culture medium containing 10% fetal bovine serum cells were seeded in a petri dish, gently pipetted to mix, and cultured at 37°C under 5% CO2 saturated humidity. When the cell density reaches 80%, discard the medium, add 0.25% trypsin to digest the cells, quickly discard the trypsin, add the complete medium, pipette the cells to make a single cell suspension, and passaged the culture at a ratio of 1:3, expanded cultivation at 37°C and 5% CO2 saturated humidity.

2.3 Preparation of ADMS overexpressing GCP2

lentivirus overexpressing the GCP2 gene was constructed, packaged and tested by Wuhan Baffir Biotechnology Service Co., Ltd [8]. Select the 3rd generation ADMS with good growth status, with 3-5×10^4 cells per milliliter, inoculate the suspension with a volume of 90ul into a 96-well plate, and co-culture with the lentivirus carrying the GCP2 gene for 48-72h. Observe the distribution of green fluorescent protein under a fluorescence microscope to determine the optimal multiplicity of infection and transfection efficiency.

2.4 Flow cytometry surface antigen detection GCP2-AMDS

Cells in the logarithmic growth phase and grow well were collected, each tube contains 1ml 0.5% bovine serum albumin (BSA) PBS, and antibodies CD29-APC and CD45-PE were added[8]. Set a negative control group at the same time, protected from light after incubating for 30 minutes, wash twice with PBS containing 0.5% BSA; after resuspending with PBS, flow cytometry detect and analysis.

2.5 GCP2-ADMS function test

GCP2-ADMS migration experiment : Cells in the logarithmic growth phase and grow well were collected, centrifuge, remove the supernatant, and rinse off the residual serum with PBS; resuspend the cells in a serum-free medium, count on a cell counting plate, and dilute the cell concentration to 3×10^5cell/ml with serum-free medium, and set aside; add 10% FBS medium (containing double antibodies) to the 24-well plate, and put the transwell chamber into it, add cell suspensions of each group to the upper chamber of the transwell, and cultrue for 24 hours in the incubator; remove the transwell and wash the chamber carefully with PBS Once, fix the cells with 70% ice ethanol solution for 1h; stain with 0.5% crystal violet staining solution, place at room temperature for 20 minutes, wash with PBS, wipe clean the non-migrated cells on the side of the upper chamber with a clean cotton ball， observe the cells and take pictures under the microscope [8].

Tube experiment of GCP2-ADMS : Collect cells in logarithmic growth phase and in good growth condition. After trypsin digestion, a single cell suspension is prepared with serum-free medium,after counting, the cells are evenly seeded into 24-well platesthe pre-lined with matrix gel according to 1.5×10^5 /cells per well, and incubated overnight for 8-12 hours， observed the cells and taked pictures under the microscope [8].

2.6 Detection of GCP2 mRNA and protein expression levels in GVP2-ADMS

Q-PCR detection of eNOS, NOX2 and NOX4 gene expression in GC P2-ADMS and ADMS

The total RNA of ADMS and GCP2-ADMS were extracted and reversed into cDNA for real-time reverse transcriptase polymerase chain reaction[8] . Ang-1 primer sequence: F: GGACAGCAGGAAAACAGAGC; R: GC CCTTTGAAGTAGTGCCAC. eNOS primer sequence: F: CCTGACAA CCCCAAGACCTAC; R: TAACATCGCCGCAGACAAAC. TNF-a pri mer sequence: F: TCAGAGGCCTGTACCTCAT; R: GGAAGACCCCT CCCAGATAG. FGF-2 primer sequence: F: AGAGCGACCCTCACAT CAA; R: CGTTTCAGTGCCACATACC. VEGF-A primer sequence: F: GGAGGAGGGCAGAATCATCA; R: CTTGGTGAGGTTTGATCCGC . SDF-1 primer sequence: F: GCTGGTCCTCGTGCTGAC; R: TCCACT TTAGCTTCGGGTCA.

Western Blot detects the expression level of GCP2 in GCP2-ADMS and ADMS : The total protein of ADMS and GCP2-ADMS were extracted with lysate and BCA was quantified, incubated with GCP2 antibody (1:1000) overnight, and β-actin (1:500) was selected as the internal control.

2.7 Animals: Experimental animals are provided by Liaoning Changshe ng Biotechnology Co., Ltd. (License No.: SCXK (Liao) 2020-0001) [8].

The rats were randomly divided into the following 4 treatment groups (n=10): control group (A group), MCT-induced PAH group (B group), ADMS treatment group (C group), and GCP2-ADMS treatment group (D group). Rats in A group were raised in normal condition. Rats in B, C, D groups were induced to pulmonary hypertension by injection of MCT at 50mg/kg for 2 weeks. At the same time, cells(ADMS 1X10^7 IU VS. GCP2-ADMS 1X10^7IU） were injected into the tail vein in groups C, D and physiological saline was injected in groups A and B, once a week for a total of 2 times in total.

2.8 Pulmonary blood pressure measurements

Two weeks after the last treatment of cells therapy, pulmonary artery pressure was measured in anesthetic rats by 7% chloral hydrate via peritoneal injection. A ployethylene cannula filled with 2% heparin sodium was inserted into the right external jugular vein and forwarded to the right ventricular (RV). The data was recorded after stabilization of the tracing using a liquid pressure transducer, which was interfaced to a Medlab electrophysiolograph [8].

2.9 Index of right ventricular hypertrophy measurements

After the rats were sacrificed,the heart was removed to determine the extent of right ventricular hypertrophy from the ratio of right ventricle to left ventricle plus septum weight (RV/LV+S) [8].

2.10 Histopathologic analysis

The lung and heart was washed by 0.1%PBS and physiological saline of 4 ℃ repeatedly. Then fixed in 10% formalin solutions overnight and em bedded in paraffin.For identification of vascularity and the thickness of p ulmonary arteriolar, formalin-fixed paraffin-embedded lungs were sectio ned, deparaffinized, stained for elastic lamina by Hematoxylin and eosin (HE). We calculated the thickness index (TI) and area index (AI) of pulm onary arterioles （diameter < 500 μm） wall by computer image analysis system (light microscopy, ×200) (selected 5 cross-sections for each secti on) [8].

2.11 Western Blot detection of GCP2, VEGF，HGF，α-actin and e NOS protein expression in lung, heart tissue of rats in each group

Selected β-actin (1:500) as the internal reference[8]. Used the lysate to extract the total protein of each group of lung tissues and perform BCA quantification, electrophoresis, membrane transfer, and color development according to the kit instructions. GCP2, VEGF, HGF, α-actin and eNOS antibody (1:1000) were incubated overnight. The gray values of GCP2/β-actin, VEGF /β-actin, HGF/β-actin, α-actin/β-actin, and eNOS/β-actin represent the relative expression of GCP2, VEGF, HGF, α-actin and eNOS protein, respectively.

2.12 Q-PCR detection of AngII, Apelin gene expression levels in lung tissue of rats in each group

The total RNA in each group of lung tissues was extracted and reversed into cDNA for real-time reverse transcriptase polymerasechain reaction. AngII primer sequence: F: TGCT GAGAGTATTGGCTGGGCAAC; R: ATGCTACTGATTTTGCCCGC. Apelin primer sequence: F: AATCTGAGTTTCTGCGTGCA; R: CTGGTCCAGTCCTCGAAGTT [8].

2.13 ELISA test

ELISA detected the expression of TNFα, IL-6, IL-10, IL-17, IL-23 in the serum of rats in each group.The blood samples of each group were centrifuged for 10 minutes, and the supernatant was collected for further investigation [8].

2.14 Statistical analysis

All quantitative data were expressed as mean ± standard deviation(SD).To compare values between these groups,analysis of variance (AVOVA) were applied.The statistical significance level was defined as p<0.05.Analyses were performed using SPASS 17.0 [8].

3.Results

3.1 optimal infection multiple and transfection efficiency of ADMS overexpressing GCP2; Flow cytometry analysis

The optimal infection multiple ratio was 4.25 times, which was significantly higher than Group ADMS. The transfection efficiency was 52%, which was also remarkably higher than that of Group ADMS (Figure 1A) ($p < 0.05$). According to the FSC-SSC scatter plot, the size and particle size of ADMS and GCP2-ADMS were mostly clustered in one area, indicating that transfection of GCP2 would not affect the original cell properties of ADMS. Antibodies CD29-APC and CD45-PE were used to screen out ADMS and GCP2-ADMS. The scatterplots showed that all the cells were clustered with little cell debris (Figure 1B) ($p < 0.05$).

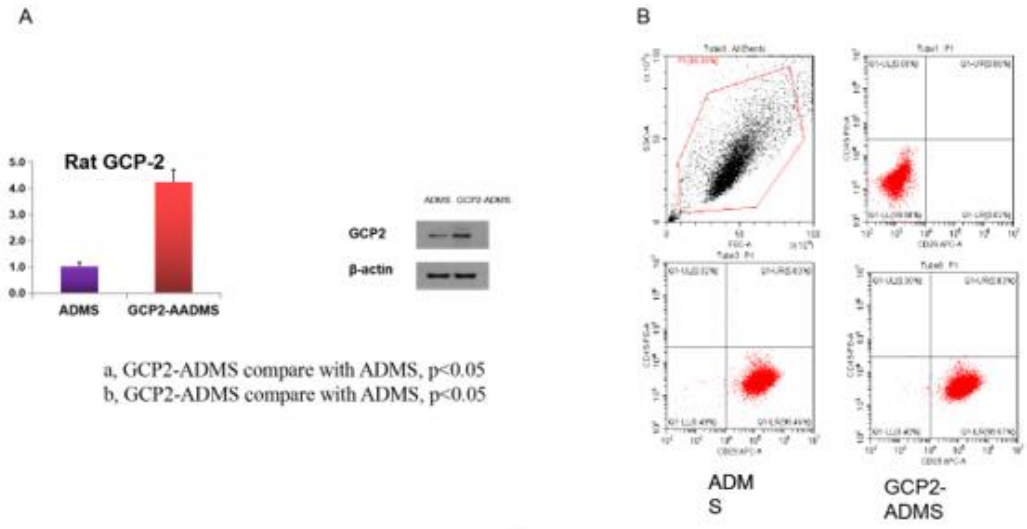


Figure 1. Transfection efficiency of ADMS overexpressing GCP2. There are two groups, one is Group ADMS, and other is Group GCP2-ADMS. [a] $p < 0.05$ compare with ADMS group; [b] $p < 0.05$ compared with ADMS group. Flow analysis results. There are two groups, one is Group ADMS, and other is Group GCP2-ADMS. Different plots represent different results, as detailed in our results section.

3.2 GCP2-ADMS migration experiment; The tube experiment; PAEC-GCP migration experiment

The migration ability of Group ADMS overexpressing GCP2 was significantly enhanced than that of Group ADMS, with a specific increase of 1.31 times (Figure 2A) ($p < 0.05$). The tube forming ability of Group GCP2-ADMS was remarkably stronger than that of Group ADMS, which increased by 78% (Figure 2B) ($p < 0.05$). The migration ability of Group PAEC overexpressing GCP2 was significantly enhanced than that of Group PAEC (Figure 2C) ($p < 0.05$).

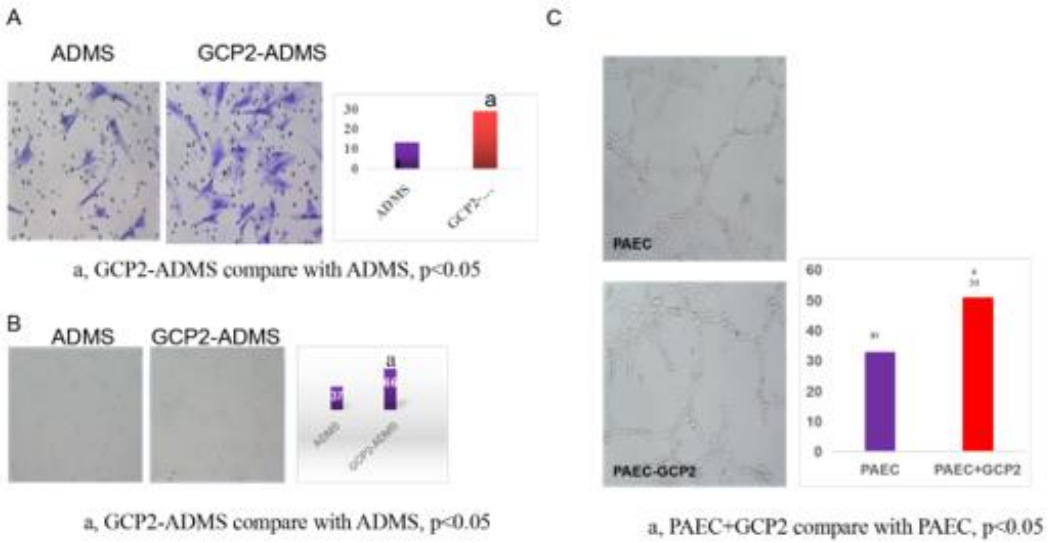


Figure 2. Migration experiment. There are two groups, one is Group ADMS, and other is Group GCP2-ADMS. [a] $p < 0.05$ compare with ADMS group. Tube experiment. There are two groups, one is Group ADMS, and other is Group GCP2-ADMS. [a] $p < 0.05$ compare with ADMS group. Migration experiment. There are two groups, one is Group PAEC, and other is Group PAEC-ADMS. [a] $p < 0.05$ compare with PAEC group.

3.3 GCP2-ADMS increased eNOS expression, by contrast, GCP2-ADMS suppressed NOX2 and NOX4 expression

First of all, we observed the QPCR- eNOS in GCP2-ADMS and ADMS. It can be seen that the eNOS geen expression was significantly different. Compared with ADMS, the eNOS geen expression in the GCP2-ADMS was remarkably increased. GCP2-ADMS supplementation significantly down-regulated the geen expression of NOX2 and NOX4 (Figure 3A) ($p < 0.05$). In the western-blotting, NOX2 and NOX4 expression was dramatically decreased in GCP2-ADMS, and eNOS expression was significantly lighted by GCP2-ADMS (Figure 3B) ($p < 0.05$).

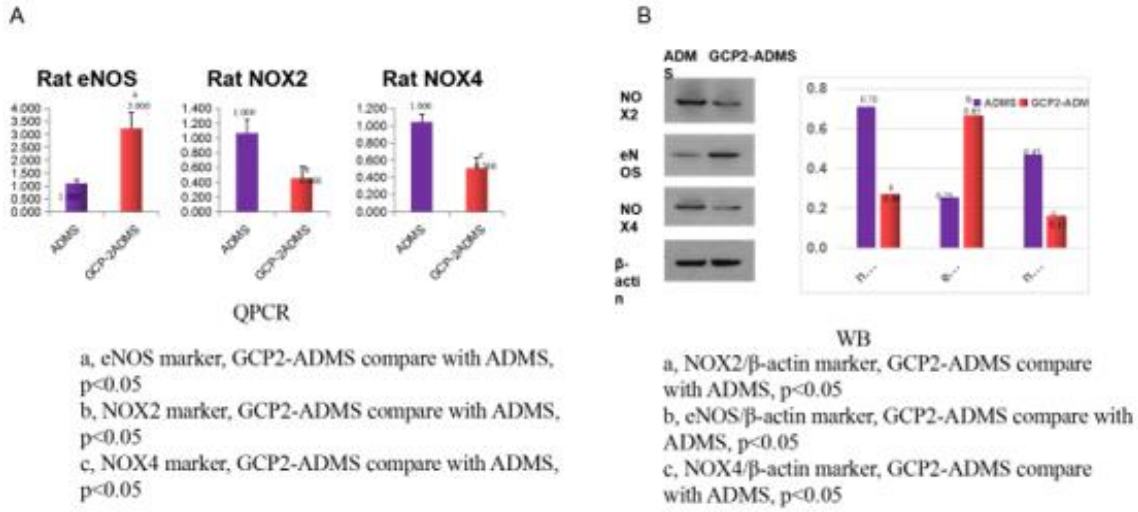


Figure 3. Q-PCR detection results of eNOS, NOX2 and NOX4 gene expression. There are two groups, one is Group ADMS, and other is Group GCP2-ADMS. [a] $p < 0.05$ meant that there was a difference between the GCP2-ADMS group and the ADM2 group in measuring eNOS. [b] $p < 0.05$ meant that there was a difference between the GCP2-ADMS group and the ADM2 group in measuring NOX2. [c] $p < 0.05$ meant that there was a difference between the GCP2-ADMS group and the ADM2 group in measuring NOX4. Western Blot detection results of eNOS, NOX2 and NOX4 protein expression. There are two groups, one is Group ADMS, and other is Group GCP2-ADMS. [a] $p < 0.05$ meant that there was a difference between the GCP2-ADMS group and the ADM2 group in measuring NOX2. [b] $p < 0.05$ meant that there was a difference between the GCP2-ADMS group and the ADM2 group in measuring eNOS. [c] $p < 0.05$ meant that there was a difference between the GCP2-ADMS group and the ADM2 group in measuring NOX4.

3.4 GCP2-ADMS exocrine cytokines, some of which promote cell proliferation and some of which inhibit inflammation

Cytokines VEGF-A, IGF, AKT-1 and IL-8 levels were the highest in the GCP2-ADMS group, while the GCP2-ADMS group showed significantly reduced TNF-α levels ($p < 0.05$) (Figure 4) ($p < 0.05$).

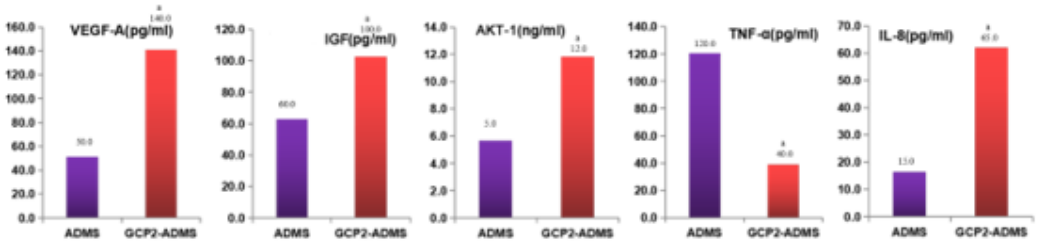


Figure 4. ELISA results of VEGF-A, IGF, AKT-1, IL-8 and TNF-α. There are two groups, one is Group ADMS, and other is Group GCP2-ADMS. [a] $p < 0.05$ compare with ADMS group.

3.5 Histopathologic analysis results

The lung tissues of the four groups of rats (Normal, PAH, ADMS and GCP2-ADMS) showed obvious inflammatory hyperplasia. The inflammation of lung tissue of GCP2-ADMS was almost completely dissipated, and the anti-inflammatory effect of GCP2-ADMS was stronger than that of ADMS. The same was true for heart tissue, where GCP2-ADMS had the most pro-proliferative and anti-inflammatory effects (Figure 5) ($p < 0.05$).

A

	Normal	PAH	ADMS	GCP2-ADMS	P
MPAP	18.0±0.96	31.50±0.70	26.80±0.68	21.70±1.48	<0.01

B

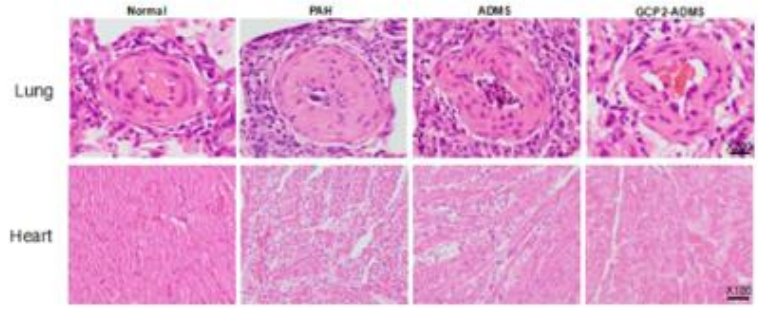


Figure 5. Analysis of pathological staining results. (A) There are four groups, Normal, PAH, ADMS and GCP2-ADMS. There was statistical difference in all groups, $p < 0.05$. (B) Endothelial cell proliferation and inflammatory cell infiltration in heart tissue and lung tissue were different in the four groups.

3.6 GCP2-ADMS increased GCP2 expression, but inhibited VEGF, HGF and α-actin expressions in pulmonary artery, heart and lung tissue

In the western-blotting results of pulmonary artery tissue, we saw the highest content of GCP2 in the GCP2-ADMS group, while the other three proteins were reduced in the GCP2-ADMS group. These results indicate that GCP2-ADMS can promote the secretion of GCP2 to repair the injured pulmonary artery, and at the same time, it can avoid the transitional proliferation of vascular tissue and maintain the dynamic balance of cell proliferation (Figure 6A) ($p < 0.05$). In the western-blotting results of pulmonary artery tissue, we revealed the highest content of Aplin and eNOS in the GCP2-ADMS group, while the other protein were lowered in the GCP2-ADMS group. In suggested that GCP2-ADMS can promote pulmonary artery relaxation and inhibit vascular inflammation (Figure 6B) ($p < 0.05$). In heart tissue, we showed that the lowest content of VEGF, HGF and α-actin in the GCP2-ADMS group, while the GCP2 protein were best in the GCP2-ADMS group. This showed that GCP2-ADMS has same effects in heart tissue (Figure 6C) ($p < 0.05$). In heart tissue, we found that the lowest level of MCP-1 in the GCP2-ADMS group, while the Apelin and eNOS protein were best in the GCP2-ADMS group. This introduced that GCP2-ADMS has simliar effects in heart tissue (Figure 6D) ($p < 0.05$). We showed that the lowest content of VEGF, HGF and α-actin in the GCP2-ADMS group, while the GCP2 protein were lightest in the GCP2-ADMS group. This showed that GCP2-ADMS has same effects in lung tissue (Figure 6E) ($p < 0.05$). In lung tissue, we saw that the lowest level of MCP-1 in the GCP2-ADMS group, while the Apelin and eNOS protein were best in the GCP2-ADMS group. This indicated that GCP2-ADMS has simliar effects in lung tissue (Figure 6F) ($p < 0.05$). These results showed that GCP2-ADMS has positive effects in all three tissues.

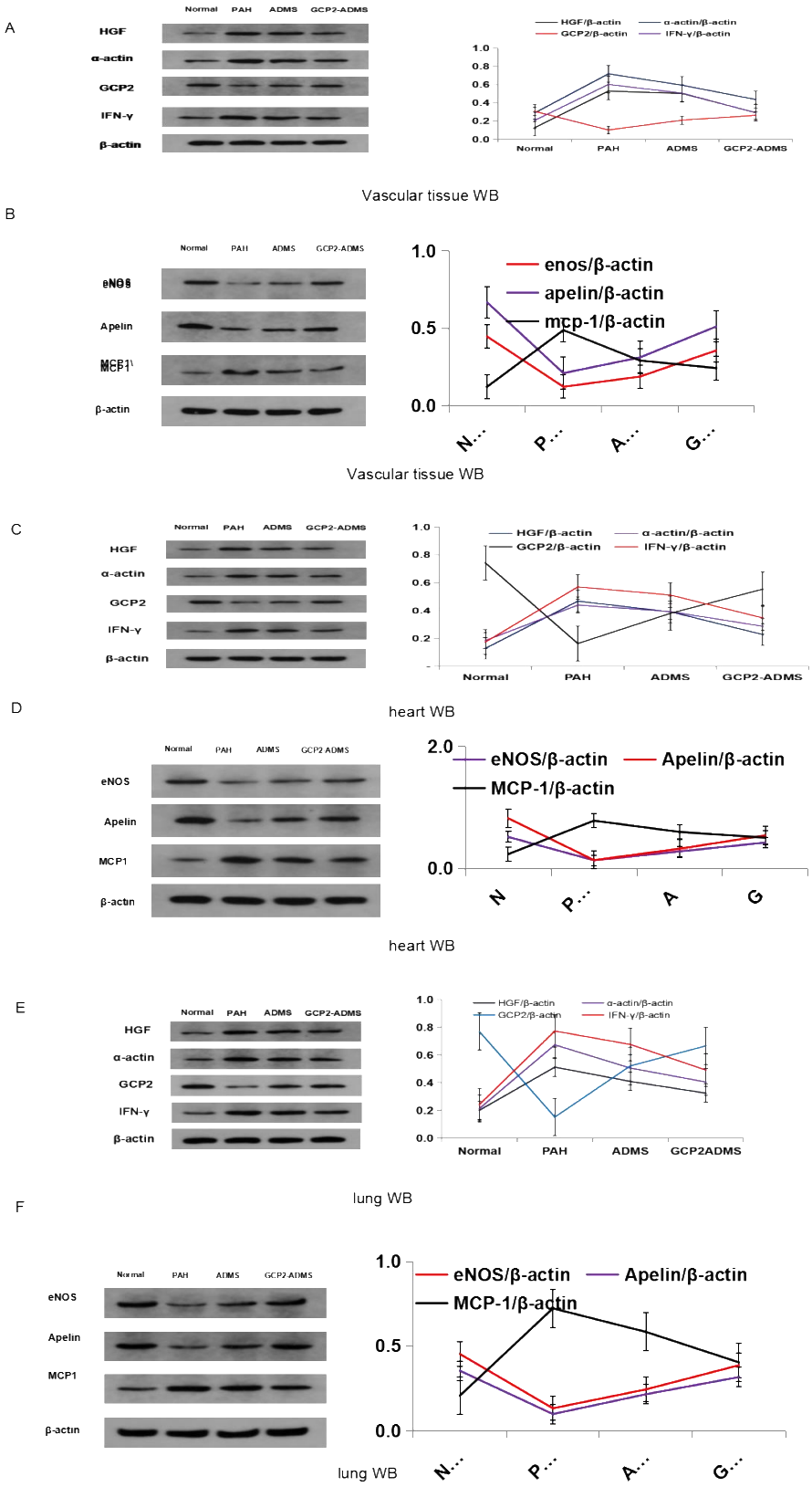
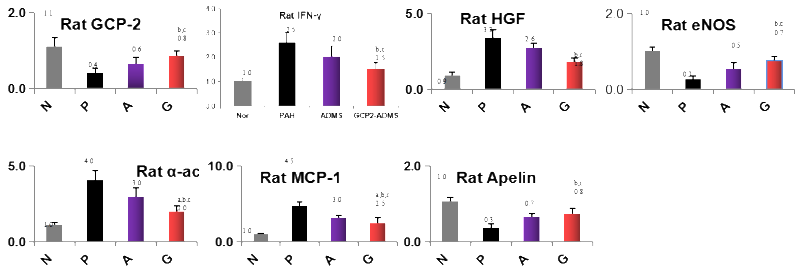


Figure 6. Quantitative results of pulmonary artery, heart, lung tissue protein. There are four groups, Normal, PAH, ADMS and GCP2-ADMS. Western blotting showed the depth of bands of HGF, α-actin, GCP-2 and IFN-γ in the four groups, respectively. The line plot shows the difference in the concentration of the four histones. Quantitative results of pulmonary artery tissue protein. There are four groups, Normal, PAH, ADMS and GCP2-ADMS. Western blotting showed the depth of bands of eNOS, Apelin and MCP-1 in the four groups, respectively. The line plot shows the difference in the concentration of the four histones.

3.7 GCP2-ADMS increased GCP2, eNOS and Apelin gene expressions, by contrast, GCP2-ADMS suppressed VEGF, HGF, α-actin and MCP-1 gene expressions in pulmonary artery, heart and lung tissue

In the lung tissue of four groups, compared with ADMS group, GCP2-ADMS group had stronger ability to express GCP2, eNOS and Apelin gene and weaker ability to express VEGF, HGF, α-actin and MCP-1 gene. This is consistent with the WB results of GCP2-ADMS group. These results indicated that GCP2-ADMS group does have certain ability to promote cell regeneration and repair, avoid excessive proliferation, anti-inflammatory nad vasodilate (Figure 7A) ($p < 0.05$). In the heart tissue of four groups, compared with ADMS group, GCP2-ADMS group had stronger ability to express GCP2, eNOS and Apelin gene and worst ability to express VEGF, HGF, α-actin and MCP-1 gene (Figure 7B) ($p < 0.05$). Four groups, GCP2-ADMS group had stronger ability to express GCP2, eNOS and Apelin gene, compared with ADMS group. And worst ability to express VEGF, HGF, α-actin and MCP-1 gene in the GCP2-ADMS group (Figure 7C) ($p < 0.05$). These results were all presented in pulmonary artery tissue.

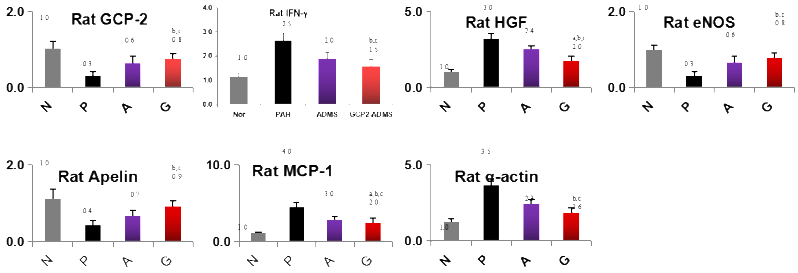
A



vascular tissue PCR

a, GCP2-ADMS compare with Normal, $p<0.05$
b,GCP2-ADMS compare with PAH, $p<0.05$
c, GCP2-ADMS compare with ADMS, $p<0.05$

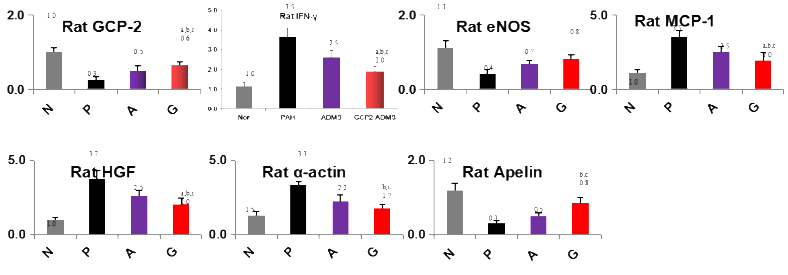
B



heart PCR

a, GCP2-ADMS compare with Normal, $p<0.05$
b,GCP2-ADMS compare with PAH, $p<0.05$
c, GCP2-ADMS compare with ADMS, $p<0.05$

C



Lung PCR

a, GCP2-ADMS compare with Normal, $p<0.05$
b,GCP2-ADMS compare with PAH, $p<0.05$
c, GCP2-ADMS compare with ADMS, $p<0.05$

Figure 7. Q-PCR results of pulmonary artery, heart, lung tissue. There are four groups, Normal, PAH, ADMS and GCP2-ADMS. [a] $p < 0.05$ compare with Normal group; [b] $p < 0.05$ compared with PAH group. [c] $p < 0.05$ compare with ADMS group.

Discussion

In our study, we found that GCP2-ADMS has stronger migration and tube forming effects. These properties may be attributed to the promotion of the eNOS gene and protein expression and the inhibition of the NOX2 and NOX4 genes as well as protein expressions. In addition, GCP2-ADMS also secretes cytokines that promote cell proliferation and are anti-inflammatory. These abilities were shown in pathological sections as significantly increased vascular cells and significantly reduced inflammatory cell infiltration in GCP2-ADMS. At the same time, GCP2-ADMS can also directly promote the production of angiogenic factors GCP2, Aplin and eNOS in pulmonary artery cells, lung cells and heart cells, and prevent cell hyperplasia as well as the spread of inflammation. In addition to these effects at the protein level, these effects also occur at the gene level.

Previous studies have shown that GCP2 has strong proliferative function, while ADMS can also promote cell generation. When genes with proliferative characteristics are transfected on ADMS, ADMS can show stronger cell migration and proliferation ability [1,5,10,11]. Interestingly, Our results are consistent with those of our predecessors. Similarly, we found that the proliferative properties of GCP2 are associated with up-regulation of eNOS and down-regulation of NOX2 and NOX4. eNOS gene is a member of a gene family, and many studies have shown that eNOS gene can significantly promote cell proliferation and migration [12-15]. Similarly, NOX2 and NOX4 genes are important members of the The NADPH oxidase family. They are in charge of cell apoptosis and inhibit cell proliferation. These characteristics play an important role in the prevention of tumor formation, but they are also easy to cause the excessive consumption of cells [16-18]. The important role of GCP2 is closely related to the regulation of these genes.

The GCP2 gene has a strong function of promoting paracrine, which coincides with our results. Our study found that the cell migration and tubular formation ability of GCP2-ADMS was significantly stronger than that of pure ADMS. This may be due to the fact that part of the gene sequence of GCP2 is related to the mechanism of regulating cell secretion [19-21]. Cells supplemented with GCP2 can promote the secretion of proliferation-promoting cytokines, such as VEGF-A, bFGF/FGF2, TGF-β1 and IL-8. These cytokines have been shown to be associated with cell proliferation and have a strong effect on vascular cells. Our results also indicate that GCP2 cells have higher levels of these cytokines and more obvious cell proliferation [22-25]. VEGF-A is closely related to the generation of vascular endothelial cells. When the level of VEGF-A increases, the anti-apoptotic ability of vascular endothelial cells will be significantly increased, and then synergistically promote the proliferation of vascular endothelial cells [26,27]. Similarly, IGF is also related to cell proliferation, which can promote the proliferation of a variety of tissues [28,29]. Some studies have shown that AKT-1 is related to cell proliferation, distributed in most tissues of the body, and regulates cell proliferation function [30,31]. And cytokine IL-8 is more well known. It is an important cytokine regulating the balance between hyperplasia and apoptosis of the body, and it exists in most cells of the body and plays a powerful role [32,33]. In contrast, we found that GCP2-ADMS inhibited the spread of inflammation, and GCP2-ADMS inhibited the progression of inflammatory cytokines IGF and AKT-1. This may be because part of the gene sequence of GCP2 coincides with inflammatory factors, indicating that GCP2 can not only promote cell proliferation, but also significantly inhibit the effect of inflammation on cell proliferation [34,35]. IGF is a downstream reaction factor of the Toll-like receptor (TLR) family. When the body reacts to the stimulatory ligand LPS, it will activate the TLR pathway, and then activate the inflammatory response of the body, promote scar hyperplasia, and inhibit cell proliferation [36-38]. AKT-1 is activated by a cellular pathway dependent on phosphatidylinositol 3 kinase (PI3K), and its oversecretion inhibits normal cell proliferation and induces malignant proliferation such as that of tumor cells [39-41].

Interestingly, GCP2-ADMS can promote cell proliferation and play an anti-inflammatory role through parasecretion, and tissue cells that receive signals regulated by GCP2 can also consciously balance the hyperplasia under the premise of normal proliferation and avoid cell transformation into tumor cells [42-45]. Similar to our results, this proves that GCP2 can not only delay the remodeling of lung vascular cells and promote cell regeneration, but also affect the hyperplasia of cardiomyocytes and lung smooth muscle cells. Increased expression of GCP2 can promote cell proliferation, while decreased expression of VEGF-A can avoid of cell proliferation. This is because previous studies have shown that VEGF-A increases in eyes tumors [46,47]. However, HGF levels in certain tumors are significantly upregulated [48,49]. The α-actin expression is significantly increased in certain tumors [50,51]**.** At the same time, our study found that GCP2-ADMS can also promote the expression of eNOS and Apelin in tissue cells. Studies have shown that Apelin gene is significantly related to cell proliferation. When cells need to proliferate, Apelin gene belongs to the first batch of genes activated, regulating cell proliferation and repair [52,53]. Similarly, eNOS gene has also been shown to be closely related to the regulation of cell proliferation and anti-cell apoptosis. As a kind of NOS enzyme, the body mainly activates VEGFR2/Akt/eNOS pathway to promote downstream eNOS gene expression and thus regulate cell proliferation and apoptosis [54,55]. Our results found that the inflammatoru factor MCP-1 was significantly reduced in tissue cells, which is related to the anti-inflammatory promotion of GCP2. MCP-1 has been shown to be closely related to vascular endothelial inflammation. In patients with pulmonary arterial hypertension, MCP-1 content is significantly elevated, and inhibition of MCP-1 content is helpful to improve vascular remodeling [56,57]. These results are closely related to previous studies.

The limitation is that our study needs to be further expanded in sample size. However, the sex of the rats was all male, and there was no further explanation of female for this kind of study.

Conclusion

In conclusion, our study confirmed that transfection of GCP2 on ADMS can promote cell proliferation, anti-inflammatory, and balance cell proliferation and apoptosis. This is a promising project that can be considered for further clinical study in the future.

Contributor form

Wen Wen, Conceptualization; Data curation; Formal analysis; Writing - original draft

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Conflict of Interest

The authors declare no competing financial interest.

Funding sources

This work was supported by grants from the Project of Guangdong Science and Technology Department (No. 10202H20200035) and Project of Zhanjiang Science and Technology Bureau (1003kzkjj20200004).

Acknowledgement

All authors who contributed to this article are stated in the author contributions, and no other individuals or organizations need to be listed in the acknowledgements section.

Data availability description

With the consent of the corresponding author, the corresponding author is willing to share the experimental data related to the paper.

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DOI: 10.31579/2692-9406/177

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