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Mfn2 inhibits chronic rejection of the rat abdominal aorta by regulating TGF-β1 levels

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ABSTRACT

Objectives: The various forms of chronic rejection share a common histological appearance termed allograft arteriosclerosis. In the early stages thereof, apoptosis of vascular smooth muscle cells (VSMC) is obviously reduced, associated with vascular intimal thickening. High-level expression of the *HSG/Mfn2* gene promotes apoptosis of rat VSMC. However, the role and mechanism of Mfn2 in inhibition of chronic allograft rejection have not been described.

Methods: In the present study, we transfected transplanted abdominal aortas of donor Lewis rats with an Mfn2-encoding or control lentivirus. And then We transplanted the donor aortas to the corresponding aortal positions in recipient rats. Transplanted aortas were collected on days 30, 60, and 90 and Masson stained to measure intimal thicknesses. Immunohistochemistry would be used to confirm TGF-β1, Mfn2 and TGF-β-R2 expression in different groups.

Results: Our results confirm that high-level expression of Mfn2 lowers the expression of TGF-β1, reduces the intimal thickness of transplanted rat abdominal aorta, and retards the process of chronic rejection.

Conclusion: Mfn2 influences TGF-β/smad pathway and may function as potential chronic rejection inhibitor.

1. Introduction

In recent years, continuous improvements in technology and organ transplantation have reduced the incidence of acute rejection, but chronic rejection still threatens the health and long-term survival of transplanted patients. Chronic rejection develops progressively and is generally irreversible; such rejection is the leading cause of late loss-of-function of transplanted livers and kidneys, and manifests as gradual deterioration of transplanted organs or tissues [1,2]. Several possible mechanisms of chronic rejection have been suggested, but consensus remains elusive [3–5]. Currently, it is considered that the most important manifestations of chronic rejection are transplant arteriosclerosis, vascular intimal hyperplasia, and solid organ fibrosis [6–8]. The intimal hyperplasia is associated principally with migration of transplanted middle artery smooth muscle cells to the endangium [9], and production of cytokines, such as transforming growth factor β1 (TGF-β1), that significantly promote the migration of smooth muscle cells [10].

Obvious fibrosis in solid organs is a pathological manifestation of chronic rejection, and TGF-β1 is the most important cytokine in this context [10]. TGF-β1 exhibits many biological activities, being involved in cell proliferation, apoptosis, tumor development, and promotion of extracellular matrix (ECM) production by regulating the expression of platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), both of which play important roles in chronic rejection [11]. The correlation between TGF-β1 synthesis and chronic rejection has been recognized by the vast majority of scholars, especially those who study kidney transplantation.

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Transplant arteriosclerosis is the principal feature of chronic rejection [8]. Chronic rejection models featuring animal solid organ transplantation have many defects, including difficult operative steps and short survival times. Fishman established the carotid artery balloon injury model in 1975; Clowes et al. [12] the carotid striping model in 1983; and Fingtrie the endothelial-type slight-damage model in 1983 [13]. However, these models feature mechanical injury, and do not explore immune responses developing after transplantation. In 1991, Mennander et al. [14] were the first to propose rat abdominal aorta transplantation as a simplified model of chronic rejection. The transplant arteriosclerosis associated with this model has three major characteristics: middle-layer cell necrosis and migration, intimal hyperplasia, and inflammatory cell infiltration. These features are very similar to those noted on development of transplant arteriosclerosis preceding chronic rejection in humans, and the model affords the advantages of a simple operative technique and prolonged survival; it has become the classic model of chronic rejection.

Mitochondrial fusion protein 2 (Mfn2), which is the product of the proliferation inhibition gene (HSG) encoded on chromosome 1 p22.3, participates in mitochondrial fusion and maintains the stability of mitochondrial structure [15,16]. Mfn2 participates in mitochondrial apoptotic pathways, potentially promoting apoptosis [17,18]. Mfn2 inhibits proliferation of vascular smooth muscle cells (VSMC), and high-level expression of *HSG/Mfn2* promotes rat VSMC apoptosis. A common histological finding upon chronic rejection triggered by graft atherosclerosis is obvious VSMC apoptosis inhibit vascular intimal thickening, but the functions of Mfn2 and changes of its expression during chronic rejection of transplanted tissue have not been explored.

In the present study, we transfected transplanted abdominal aortas of donor Lewis rats with an Mfn2-encoding or negative control (NC) lentivirus by soaking each aorta in a mixture of lentivirus-containing and UW organ preservation solutions (volume ratio: 1:1) at 4 °C for 3 days. We transplanted the Mfn2-transfected or NC donor aortas to the corresponding aortal positions in recipient BN rats. In Blank group transplanted abdominal aortas were soaked only in a mixture of physiological saline and UW organ preservation solutions (volume ratio: 1:1) at 4 °C for 3 days without lentivirus transfection. We collected transplanted aortas on days 30, 60, and 90 and used Masson staining and ImagePro-Plus version 6.0 software to measure intimal thicknesses. The thicknesses of Mfn2-transfected abdominal aortas were less than those of the Blank and NC groups ($p < 0.001$) on days 60 and 90. Immunohistochemistry confirmed that the expression levels of TGF-β1 and TGF-β-R2 in intimae of Mfn2-transfected abdominal aortas were lower than those in the Blank and NC groups on days 60 and 90 after transplantation (both p values < 0.01), suggesting that Mfn2 may retard chronic rejection by inhibiting TGF-β1 expression.

2. Materials and methods

2.1. Establishment of a rat abdominal aorta transplantation model

Male Lewis (RT11) and BN (RT1n) rats 8 weeks of age and weighing 200 g were obtained from the Beijing Weitong Lihua company, and were raised in the laboratory animal center of the First Affiliated Hospital of Zhejiang University. Lewis rats (donors) were anaesthetized by intraperitoneal injection of chloral hydrate (1 ml of a 4 g/100 ml solution/100 g). Decubitus fixation was applied after anesthesia, the hair removed from the center of the abdomen, and sterile drapes applied after disinfection with iodine and alcohol. The abdominal cavity was exposed via an abdominal midline incision, Blunt dissection of the abdominal aorta and inferior vena cava was performed, with placement of cotton swabs between branches of the renal and iliac arteries. Vascular clamps were carefully applied to the ends of the abdominal aorta and segments about 1 cm in length removed. The vascular cavity was rinsed with a heparin solution (0.1 mg/ml) in saline, and the adventitia pruned. The aortal segments were transfected with lentivirus by soaking the tissue in a mixture of lentivirus-containing and UW organ preservation solutions (volume ratio: 1:1) at 4 °C for 3 days. Similarly, abdominal aortal segments were removed from recipient rats, which were then transplanted with transfected aortal segments via continuous suturing using 8–0 stitches. The vascular clamps were removed simultaneously and the vascular anastomoses pressed with cotton swabs for about 30 s; abdominal cavities were next closed if no bleeding was evident.

Rats were divided in 3 groups: 1) Blank group: donor abdominal aortas were soaked in a mixture of physiological saline and UW organ preservation solution (volume ratio: 1:1) at 4 °C for 3 days. 2) NC group: donor abdominal aortas were soaked in a mixture of negative control lentivirus (1 × 10 e8 TU/ml) and UW organ preservation solution (volume ratio: 1:1) at 4 °C for 3 days. 3) Mfn2 group: donor abdominal aortas were soaked in a mixture of Mfn2-encoding lentivirus (:1 × 10 e8 TU/ml) and UW organ preservation solution (volume ratio: 1:1) at 4 °C for 3 days.

The three groups each contained eight rats, and were examined at 30, 60, and 90 days. This study was performed according to the principles for the humane treatment of animals and was approved by the Zhejiang University Animal Care and Use Committee.

2.2. H&E staining

1) Preparation of paraffin-embedded tissue: Tissues were fixed in 4% (*v*/v) neutral formalin for 3 days and dehydrated by passage through a series of baths of increasing ethanol concentration (2 h per bath). The tissues were rendered transparent by soaking in dimethylbenzene twice, for 10 min each. Paraffin infiltration in a 60 °C incubator followed, and tissues were embedded in metal molds and held at room temperature or 4 °C; 2) Paraffin sectioning: Serial sections of paraffin-embedded tissue (5 μm thick) were made after warming tissue samples in a 40 °C water bath; the sections were oven-dried for 2 h; and held at room temperature or 4 °C; 3) Dewaxing: Slides were held in the dimethylbenzene I, II, and III solutions, respectively, for 8 min each; 4) Hydration: Slides were held in baths containing anhydrous ethanol (three baths); and 90%, 80%, and 70% (all *v*/v) ethanol, each for 3 min; 5) Hematoxylin staining for 13 min followed, and the slides were washed in running water; 6) Slides were dipped in alcohol containing hydrochloric acid for 5 s, and washed gently with water for 25 min; 7) Eosin staining (for about 2 min) followed; 8) Dehydration: Slides were consecutively immersed in 80%, 90% (both *v*/v), and anhydrous ethanol baths for 3 min in each bath; 9) To develop transparency, slides were immersed in dimethylbenzene I and II solutions for 5 min each, sealed with neutral resin after natural air-drying, air-dried once more, and observed using an optical microscope.

2.3. Masson staining

1) Paraffin sectioning: Serial sections of paraffin-embedded tissue (5 μM thick) were made after warming in a 40 °C water bath; the sections were oven-dried for 2 h; and held at room temperature or 4 °C; 2) Dewaxing: Slides were held in the dimethylbenzene I, II, and III solutions, respectively, for 8 min each; 3) Hydration: Slides were held in baths containing anhydrous ethanol (three baths); and 90%, 80%, and 70% (all *v*/v) ethanol, each for 3 min; 4) Slides were held in Weigert iron hematoxylin solution (Weigert iron hematoxylin A and B solutions were mixed in equal proportions) for 5–10 min, and washed gently with water; 5) Slides were dipped in alcohol containing hydrochloric acid for 5 s, and washed gently with water for 25 min; 6) Slides were treated with phosphomolybdic acid solution for about 5 min, and directly stained with aniline blue solution for 5 min, without water washing; 7) Slides were treated with 1% (*v*/v) glacial acetic acid for 1 min, and repeatedly dehydrated in 95% (v/v) alcohol; 8) Slides were dehydrated in anhydrous alcohol, rendered transparent using dimethylbenzene, sealed with neutral resin, air-dried, and microscopically observed using

an optical microscope.

After staining, collagen fibers, mucus, and cartilage were blue. Elastic fibers were brown. Muscle fibers, cellulose, and red blood cells were red. The nucleus was blue-black.

2.4. Immunohistochemistry

The procedures of the two-step immunohistochemical method were as follows: 1) Oven-dry the tissue at 60 °C for 30 min; 2) Dewax with dimethylbenzene; hydrate in a series of baths of increasing ethanol concentration; dewax in dimethylbenzene I solution for 20 min and in dimethylbenzene II solution for 20 min; hydrate in anhydrous ethanol I solution for 3 min, in anhydrous ethanol II solution for 3 min, in 95% (*v*/v) ethanol for 3 min, in 85% (v/v) ethanol for 3 min, and in 75% (v/v) ethanol for 3 min; 3) Wash in water twice for 10–15 s each; 4) Immerse the tissue in citric acid antigen repair solution, apply high-pressure heat for 3 min (until steaming commenced); 5) Cool naturally to room temperature; 6) Wash ×3 with PBS for 3 min each; 7) Incubate in 3% (*v*/v) H_2O_2 solution for 10 min at room temperature to suppress endogenous peroxidase activity; 8) Wash with PBS three times; 9) Add the first antibody (at a dilution of 1:1000) and hold overnight at 4 °C; 10) Allow to warm to room temperature over 30 min on the next morning; 11) Wash ×3 with PBS for 3 min each; 12) Add the second antibody, incubate at room temperature for 20 min; 13) Wash ×3 with PBS for 3 min each; 14) Incubate with DAB for 3 min, wash in water for 30 min; 15) Stain with hematoxylin for 2 min, wash in water for 30 min; 16) Hydrate and render transparent: Hydrate by passing samples through baths of different ethanol concentrations, for 3 min each. The order of incubations was 75% ethanol for 1 min, 85% ethanol for 1 min, 95% ethanol for 3 min (all *v*/v), anhydrous alcohol I for 1 min, anhydrous alcohol II for 1 min, dimethylbenzene I for 3 min, and dimethylbenzene II for 3 min; 17) Seal with neutral resin, air-dry, and observe under an optical microscope.

2.5. Immunohistochemical staining criteria

Vision setting 5, at high magnification, was used to randomly observe staining intensities and proportions. Grading by staining intensity was: 0 for no color, 1 for pale yellow, 2 for brownish yellow, and 3 for brown. Grading by positive cell proportion was: 0 for < 5%, 1 for 5–10%, 2 for 11–50%, and 3 for > 50%. The product of the score of color intensity and the score of positive cell is the final score: - was 0 point; + was 1, 2 points; + + was 3, 4 points; and + + + was 6, 9 points [19] (Table 1).

2.6. Statistical analysis

Experiments were performed in triplicate. Student's *t*-tests were used to assess differences between groups. Data are displayed as means ± standard deviations. $P < 0.05$ indicates statistical significance. *,** and *** represents $P < 0.05$, < 0.01 and < 0.001, respectively.

Table 1
Immunohistochemical staining criteria.

Color intensity scoring		Positive cell proportions		Product	
Color intensity	Score	Percentage	Score		
None	0	< 5	0	0 point	-
Weak (yellow))	1	5–10	1	1,2 points	+
Medium (tan)	2	11–50	2	3,4 points	+ +
Strong (brown)	3	> 50	3	6,9 points	+ + +

3. Results

We used H&E staining, Masson staining to ensure the transplanted aorta structure (Fig. 1A). After 72 h transfection of lentivirus, which can express GFP (Green Fluorescent Protein), we observed the efficiency of transfection under fluorescence microscope and it showed that the cells of aorta were transfected with lentivirus and emitted green fluorescence (Fig. 1B). Fig. 1C shows the transplanted abdominal aorta in BN rat. On day 30, 60 and 90 we collected the transplanted aorta and using Masson staining to stain the vessel (Fig. 1D). We used the ImagePro-Plus version 6.0 software to measure the intimal thickness of transplanted rat abdominal aortas (Fig. 1E). It showed that the thickness of Mfn2-transfected aortas was less than those of the Blank group and the group transfected with NC lentivirus ($p < 0.001$) on days 60 and 90, but no obvious among-group difference was evident on day 30 ($p > 0.05$). Also, the data in the Blank and NC groups did not differ from that on days 60 or 90 ($p > 0.05$) (Fig. 1E).

Immunohistochemistry confirmed the expression levels of Mfn2, TGF-β1 and TGF-β-R2 in the Blank and NC groups on day 30, 60 and 90 days after aorta transplantation (Fig. 2A-C)

Expression level of Mfn2 in Blank and NC groups on 60 days were lower than on day 30 ($p < 0.01$), but there was significant difference when these two groups compared with Mfn2 group($P < 0.001$) (Fig. 3A). In the Blank and NC groups, the expression levels of TGF-β1 and TGF-β-R2 were higher on day 60 than on day 30 ($p < 0.01$), and clearly differed from those in Mfn2 group on day 60 ($p < 0.01$); but the levels of TGF-β1 and TGF-β-R2 in the Mfn2 group on day 60 did not differ from those on day 30 (both p values > 0.05)(Fig. 3B–C). The expression levels of Mfn2 in the Blank and NC groups were maintained at low levels to day 90, and did not differ from those on day 60 (both p values > 0.05), but clearly differed from that of the Mfn2 group on day 90 ($p < 0.001$)(Fig. 3A). Expression of TGF-β1 and TGF-β-R2 in both the Blank and NC group was maintained at high levels to day 90 and did not differ from that on day 60 (both p values > 0.05), but clearly differed from those of the Mfn2 group on day 90 (both p values < 0.01). The expression levels of TGF-β1 and TGF-β-R2 in the Mfn2 group on day 90 were similar to those on day 60 (both p values > 0.05) (Fig. 3B–C).

4. Discussion

Chronic rejection is the main danger posed to the health and long-term survival of organ transplant patients. The mechanism thereof remains unclear. It is generally accepted that the principal manifestations of chronic rejection are transplant arteriosclerosis, vascular intimal hyperplasia, and fibrosis of substantive organs [1,8,14]. TGF-β1, a cytokine associated with chronic rejection, plays its role by exerting profibrogenic effects, as shown in many studies on renal transplantation [10,11]. The principal manifestations of chronic rejection are transplant arteriosclerosis and vascular intimal hyperplasia, including intermediate cell necrosis and migration, endometrial hyperplasia, and inflammatory cell infiltration. Therefore, reduction of transplant arteriosclerosis and reducing the thickness of arterial intimae are key aims requiring immediate attention.

In the present study, we used lentivirus encoding Mfn2 to successfully transfect rat abdominal aortas (the donor arteries), to create a model of chronic rejection after rat aorta transplantation. Masson staining, and measurement of the intimal thickness of transplanted rat aortas using ImagePro-Plus, showed that, at both 60 and 90 days after transplantation, the intimal thickness of aortas transfected with Mfn2 lentivirus was less than those of aortas transfected with a negative control lentivirus, and aortas of the Blank group ($p < 0.001$). However, 30 days after transplantation, intimal thickness did not differ significantly among the three groups ($p > 0.05$). This was also true of the Blank and NC groups 60 days and 90 days after transplantation. Thus, enhanced expression of Mfn2 inhibits intimal thickening of

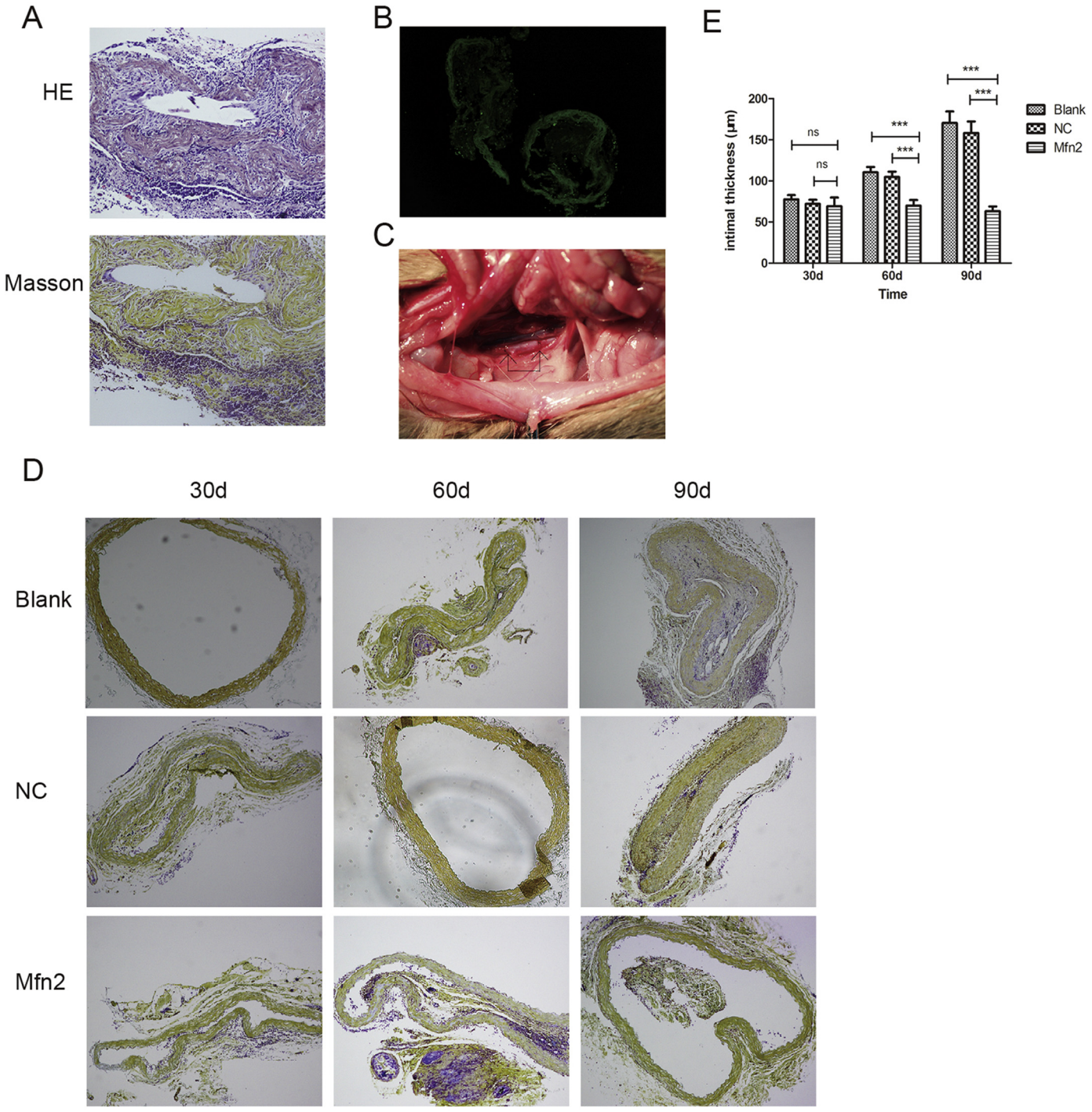

Fig. 1. A. 90 days after transplantation abdomen aorta intima HE staining and masson staining (200 x); B. Rat abdominal aorta transduced with lentivirus for 72 h and observed under fluorescence microscope (100×); C. Arrows pointed to the transplanted abdominal aorta in BN rats; D. Representative images of transplanted aorta after transplantation in rat's abdomen of Blank, NC and Mfn2 group on day 30, 60 and 90, respectively. (100×); E. The graph shows aortic intima thickness after transplantation in rat's abdomen of Blank, NC and Mfn2 group on day 30, 60 and 90, respectively.

transplanted vessels and retards chronic rejection. How does Mfn2 achieve these ends?

Current research suggests that arterial intimal thickening is actually a form of fibrotic hyperplasia, and TGF-β1 [2,10,11], which exerts a powerful profibrogenic effect, is recognized to promote chronic rejection. Therefore, we immunohistochemically explored the expression levels of Mfn2, TGF-β1, and TGF-β-R2. Thirty days after transplantation, the expression level of Mfn2 in the Mfn2 group was greater than those in the Blank and NC groups ($p < 0.05$), attributable to Mfn2-encoding lentivirus transfection prior to operation. Both TGF-β1 and TGF-β-R2 were expressed at low (and similar; both p values > 0.05) levels in all three groups. By day 60, the Mfn2 levels in the Blank and NC groups had obviously decreased (compared to day 30; $p < 0.01$), and the difference between these levels and that of the Mfn2 group was significant ($p < 0.01$). Also, TGF-β1 and TGF-β-R2 were expressed to greater extents in these two groups (compared to day 30; $p < 0.01$). The levels differed from those in the Mfn2 group (both p values < 0.01), in which the levels on days 30 and 60 were similar ($p > 0.05$).

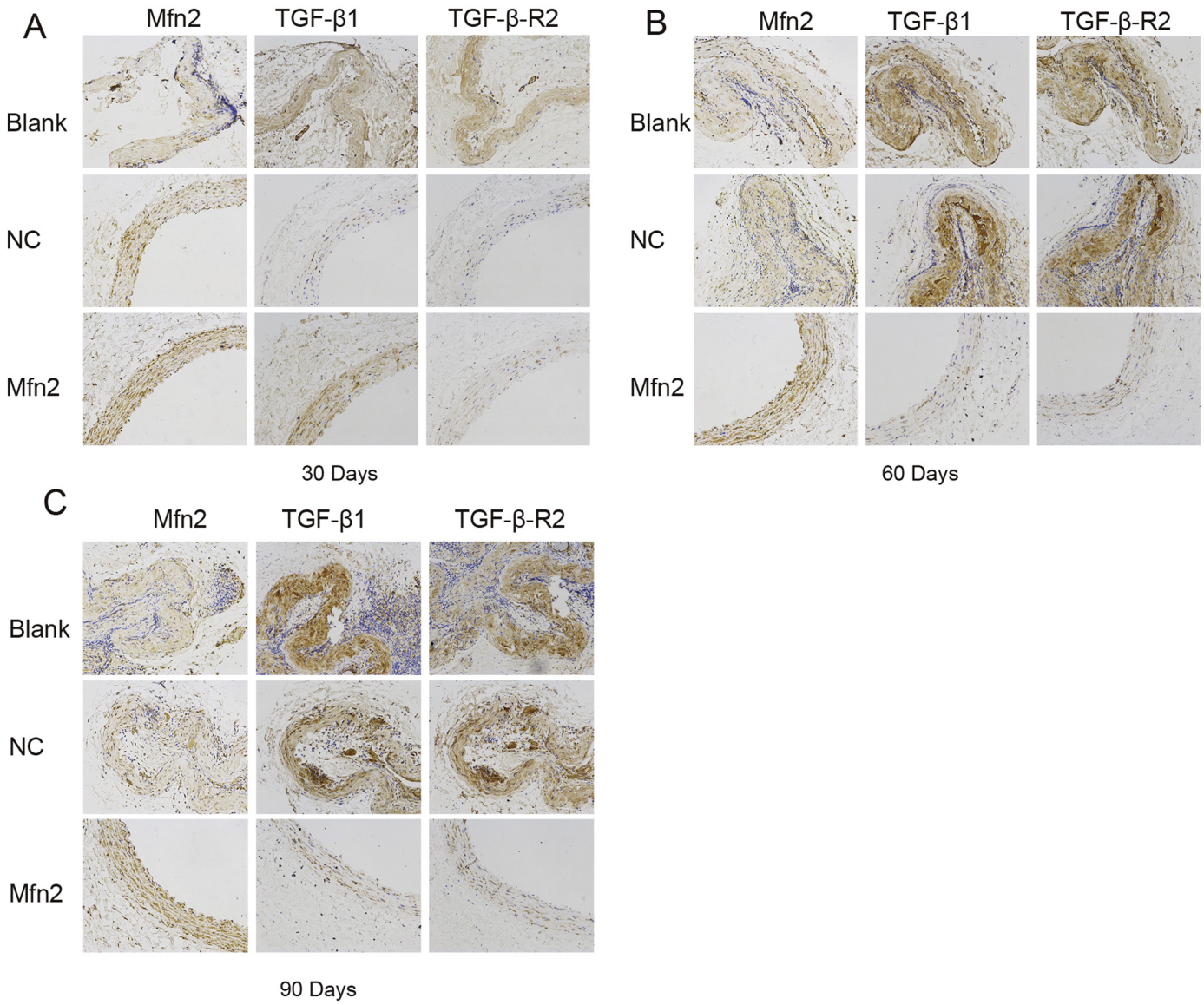

Fig. 2. A. Expression of Mfn2, TGF-β1 and TGF-β-R2 in 30 days after transplantation (200×); B. Expression of Mfn2, TGF-β1 and TGF-β-R2 in 60 days after transplantation(200×); C. Expression of Mfn2, TGF-β1 and TGF-β-R2 in 90 days after transplantation (200×).

On day 90, the expression levels of Mfn2 remained similar to those on day 60 ($p > 0.05$) in both the Blank and NC groups, but the level differed from that of the Mfn2 group ($p < 0.001$). At this time, the expression levels of TGF-β1 and TGF-β-R2 remained high in the former two groups (and were similar to the levels on day 60; both p values > 0.05). Although the expression levels of TGF-β1 and TGF-β-R2 in the Mfn2 group were also stable (both p values > 0.05), these levels differed from those in the other two groups (both p values < 0.01). These data indicated that, during the genesis and development of chronic rejection after rat abdominal aorta transplantation, high-level Mfn2 expression in the aorta inhibited expression of TGF-β1 and TGF-β-R2, thus retarding the progress of rejection. In conclusion, Mfn2 retards chronic rejection by inhibiting TGF-β1 expression, and our work identifies a possible therapeutic target for development of clinically useful anti-rejection drugs.

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Disclosure of potential conflicts of interest

the authors disclose no potential conflicts of interest.

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Q.S. and L.C. participated in the writing, editing and formatting the article. W.W. designed the research and conceived the project. Q.S., L.C. and D.Z. were responsible for rat aorta transplantation. W.S. and Q.Y. were responsible for tissue staining and semi-quantified the staining level. Q.S., D.C. statistical analyzed the staining results. All authors contributed to the data interpretation and report preparation.

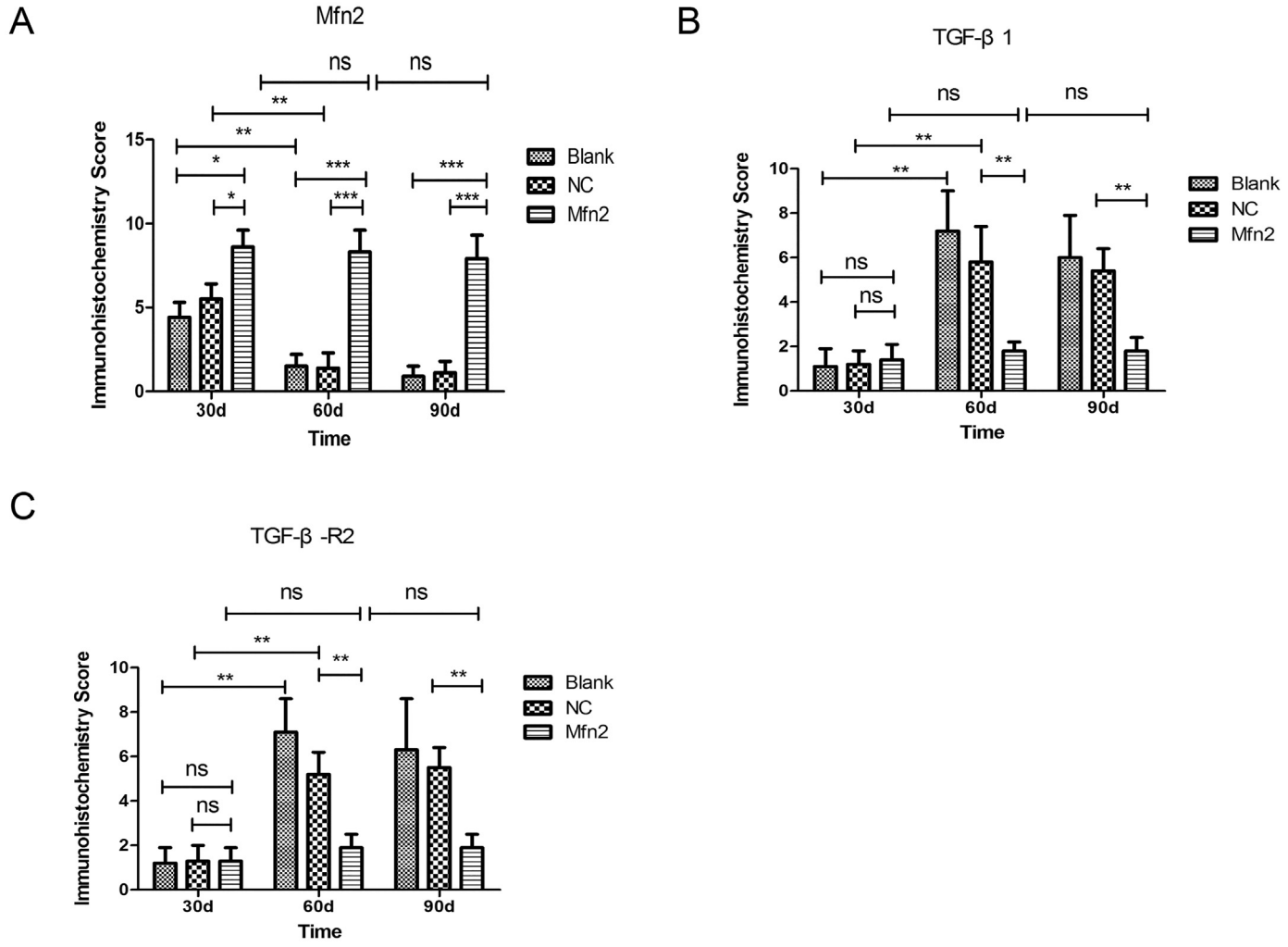

Fig. 3. A. The graph shows Immunohistochemical score of Mfn2 after transplantation; B. The graph shows Immunohistochemical score of TGF-β1 after transplantation; C. The graph shows Immunohistochemical score of TGF-β-R2 after transplantation.

References

[1] P. Libby, J.S. Pober, Chronic rejection, Immunity 14 (4) (2001) 387–397.
[2] S.A. Joosten, Y.W. Sijpkens, C. van Kooten, L.C. Paul, Chronic renal allograft rejection: pathophysiologic considerations, Kidney Int. 68 (1) (2005) 1–13.
[3] S.M. Brunner, G. Schiechl, W. Falk, et al., Interleukin-33 prolongs allograft survival during chronic cardiac rejection, Transpl. Int. 24 (10) (2011) 1027–1039.
[4] L.A. MacMillan-Crow, J.P. Crow, J.D. Kerby, J.S. Beckman, J.A. Thompson, Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts, Proc. Natl. Acad. Sci. U. S. A. 93 (21) (1996) 11853–11858.
[5] S.A. Joosten, C. van Kooten, L.C. Paul, Pathogenesis of chronic allograft rejection, Transpl. Int. 16 (3) (2003) 137–145.
[6] T.A. Wynn, Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases, J. Clin. Invest. 117 (3) (2007) 524–529.
[7] L.D. Cornell, R.N. Smith, R.B. Colvin, Kidney transplantation: mechanisms of rejection and acceptance, Annu. Rev. Pathol. 3 (2008) 189–220.
[8] S.N. Nadig, Transplant Arteriosclerosis, Transplantation. 100 (11) (2016) 2249–2250.
[9] M.A. Jansen, H.G. Otten, R.A. de Weger, M.M. Huibers, Immunological and fibrotic mechanisms in cardiac allograft vasculopathy, Transplantation 99 (12) (2015) 2467–2475.
[10] J. Li, J. Xiong, B. Yang, et al., Endothelial cell apoptosis induces TGF-beta Signaling-dependent host endothelial-Mesenchymal transition to promote transplant arteriosclerosis, Am. J. Transplant. 15 (12) (2015) 3095–3111.
[11] S. Jain, P.N. Furness, M.L. Nicholson, The role of transforming growth factor beta in chronic renal allograft nephropathy, Transplantation 69 (9) (2000) 1759–1766.
[12] A.W. Clowes, M.A. Reidy, M.M. Clowes, Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in the absence of endothelium, Lab. Investig. 49 (3) (1983) 327–333.
[13] P. Hayry, H. Isoniemi, S. Yilmaz, et al., Chronic allograft rejection, Immunol. Rev. 134 (1993) 33–81.
[14] A. Mennander, S. Tiisala, J. Halttunen, et al., Chronic rejection in rat aortic allografts. An experimental model for transplant arteriosclerosis, Arterioscler. Thromb. 11 (3) (1991) 671–680.
[15] H. Chen, S.A. Detmer, A.J. Ewald, et al., Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development, J. Cell Biol. 160 (2) (2003) 189–200.
[16] Y. Eura, N. Ishihara, T. Oka, K. Mihara, Identification of a novel protein that regulates mitochondrial fusion by modulating mitofusin (Mfn) protein function, J. Cell Sci. 119 (2006) 4913–4925 Pt 23.
[17] O.M. de Brito, L. Scorrano, Mitofusin 2 tethers endoplasmic reticulum to mitochondria, Nature. 456 (7222) (2008) 605–610.
[18] M. Karbowski, Y.J. Lee, B. Gaume, et al., Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis, J. Cell Biol. 159 (6) (2002) 931–938.
[19] E. Rosivatz, I. Becker, K. Specht, et al., Differential expression of the epithelial-mesenchymal transition regulators snail, SIP1, and twist in gastric cancer, Am. J. Pathol. 161 (5) (2002) 1881–1891.