Data Article

Data on the involvement of Meox1 in balloon-injury-induced neointima formation of rats

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Article info

Article history:
Received 3 November 2017
Received in revised form 16 November 2017
Accepted 16 November 2017
Available online 22 November 2017

Keywords:
Meox1
SMCs
Balloon-injury
Neointimal formation

Abstract

In the previous report, Meox1 was found to promote SMCs phenotypic modulation and injury-induced vascular remodeling by regulating the FAK-ERK1/2-autophagy signaling cascade (Wu et al., 2017) [1]. Here, we presented new original data on the involvement of Mesoderm/mesenchyme homeobox gene 1 (Meox1) in balloon-injury-induced neointima formation of rat. In rat carotid artery balloon injury model to induce vascular remodeling, Meox1 was induced in vascular smooth muscle cell (SMCs) of rat carotid arteries. Most proliferating cell nuclear antigen (PCNA)-positive cells also expressed Meox1. These data suggested that Meox1 may be involved in SMCs proliferation during injury-induced neointima formation. Furthermore, knocked down its expression in injured
arteries by adenoviral delivery of Meox1 short hairpin RNA (shRNA) (shMeox1), neointima formation was significantly inhibited. Elastin staining also confirmed the reduction of neointima in Meox1 shRNA-transduced arteries. Moreover, knockdown of Meox1 decreased the collagen production/deposition that was significantly increased in neointima induced by balloon injury.

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Specifications Table

| Subject area | Basic Medicine |
|--------------|----------------|
| More specific subject area | Vascular biology |
| Type of data | Figures and text |
| How data was acquired | The images were obtained from Microscope, Nikon 80i. Immunoblotting were measured with densitometry by Image J software (NIH). |
| Data format | Raw and analyzed |
| Experimental factors | The carotid arteries were fixed in 4% paraformaldehyde and embedded in paraffin, and then were cut into sections with 5 µm thickness. The segments for Western blotting were grinded in liquid nitrogen and homogenized on ice in RIPA buffer containing protease inhibitors. |
| Experimental features | H&E, Masson’s, and Verhoeff’s elastic staining were used to observe the morphology of the tissue. Western blot, IHC or IF were used to analysis the expression of Meox1 and PCNA. The immunoblots exhibited by enhanced chemiluminescence reaction were measured with densitometry by Image J software (NIH). |
| Data source location | Institute of Clinical Medicine and Department of Cardiology, Renmin Hospital, Hubei University of Medicine, Shiyan, Hubei 442000, People's Republic of China; |
| Data accessibility | The data are available with this article |
| Related research article | Mesoderm/mesenchyme homeobox gene l promotes vascular smooth muscle cell phenotypic modulation and vascular remodeling (DOI: 10.1016/j.ijcard.2017.10.098) |

Value of the data

1) The data revealed that Meox1 was induced in arterial SMCs during injury-induced vascular remodeling, and promoted injury-induced neointimal formation in rat carotid artery.
2) These data are the further evidence on the interplay between neointimal formation and Meox1 [1].
3) The data in this article provide the potential therapeutic effect of Ad-Sh-Meox1 on restenosis following percutaneous coronary intervention (PCI) through promoting re-endothelialization while inhibiting neointimal formation.

1. Data

To determine the role of Meox1 in SMCs phenotypic modulation in vivo, we used rat carotid artery balloon injury model to induce vascular remodeling. Injury induced progressive neointima formation in the arteries (Fig. 1A). Meox1 was initially induced in medial SMCs (1 and 3 days after the injury)
Meox1 expression, we performed Western blot using proteins extracted from the sham-operated control or balloon-injured arteries. As shown in Fig. 1B–C, Meox1 was time-dependently induced along with a cell proliferating marker PCNA in carotid arteries following injury. To determine if Meox1 was expressed in SMCs and if Meox1-expressing cells contributed to the SMCs proliferation, we co-stained Meox1 with smooth muscle α-actin (α-SMA) and PCNA in arteries injured for 14 days, respectively. As shown in Fig. 1D, most Meox1-expressing cells were α-SMA-positive. Most PCNA-positive cells also expressed Meox1 (Fig. 1D).

To determine if Meox1 plays a role in the injury-induced neointimal formation, we knocked down its expression in injured arteries by adenoviral delivery of Meox1 shRNA. Knockdown of Meox1 (Fig. 2A–B) significantly inhibited the neointima formation as well as the intima/media ratio by nearly 40% (Fig. 2C–D). Elastin staining also confirmed the reduction of neointima in Meox1 shRNA-transduced arteries. Moreover, knockdown of Meox1 decreased the collagen production/deposition that was significantly increased in neointima by balloon injury (Fig. 2E), which is likely due to the role of Meox1 in modulating SMCs phenotype in the neointima.
2. Experimental design, materials, and methods

2.1. Rat carotid artery injury model

According to our previous protocols for model of balloon-injury carotid artery of rat [2], the mix gas including Isoflurane (1.5–2.5%) and oxygen was used for anesthetizing rats. After introducing a 2F Fogarty arterial embolectomy balloon catheter through the external carotid artery in left, pre-filled saline in the catheter were injected with 0.02 mL to inflate the balloon, and then rotatably withdrawn through the common carotid artery from proximal to the distal end of the heart. The above procedures were repeated for 3 times to achieve the perfect balloon-injury carotid artery with the traits of utter endothelial denudation. 100 µL of saline, Ad-Null or Ad-shMeox1 was injected into the balloon-injury carotid arteries through the specific syringe (WISP SYR, Hamilton), respectively. Subsequently, in order to transduce expression adenoviral vector of the interest genes into the cells of balloon-injury carotid artery, filling saline, Ad-Null or Ad-shMeox1 were resided in these arteries for incubation of 20 min, respectively.

2.2. Histomorphometric analysis and immunohistochemistry staining

The common carotid arteries were fixed in 4% paraformaldehyde and embedded in paraffin. The sections with 5 µm thickness were stained with modified hematoxylin and eosin (H&E), Masson’s, and Verhoeff’s elastic stain (VEG) Kit, respectively. For immunohistochemistry (IHC), sections with 5 µm thickness were rehydrated, blocked with 5% horse serum and incubated with a series of primary antibodies: goat anti-Meox1 (1:100, SC-10185, Santa Cruz Biotechnology), goat anti-Meox1 (1:200, ab105349,
ABCAM), mouse anti-smooth muscle α-actin (α-SMA, 1:100, sc-130616, Santa Cruz Biotechnology), or proliferating cell nuclear antigen (PCNA) (1:200, ab29, Abcam) overnight at 4 °C followed by incubation with horseradish peroxidase. For immunofluorescent staining, fluorescein isothiocyanate (FITC) or tetra-ethyl rhodamine isothiocyanate (TRITC)-conjugated secondary antibody was used. The sections were counterstained with hematoxylin or 4,6-diamino-2-phenyl indole (DAPI).

2.3. Cell lysis, immunoblotting and antibodies

The segments for Western blotting were grinded in liquid nitrogen and homogenized on ice in Radio-Immunoprecipitation Assay (RIPA) buffer containing protease inhibitors. Cell or tissue debris was removed by centrifugation. 30 µg of proteins were separated in SDS-PAGE and were transferred to polyvinylidene difluoride membrane (Millipore). After being blocked by 5% fat free milk, the membrane was incubated with a series of primary antibodies: Meox1 (1:500, SC-10185, Santa Cruz Biotechnology), Meox1 (1:1000, ab105349, ABCAM), PCNA (1:500, sc-56, Santa Cruz Biotechnology), or β-actin antibody (1:5000, A5441, Sigma) followed by incubation with horseradish peroxidase-conjugated secondary antibodies (1:10000, Santa Cruz). The immunoblots exhibited by enhanced chemiluminescence reaction (Amersham Pharmacia Biotech) were measured with densitometry by Image J software (NIH).

2.4. Statistical analysis

All data were evaluated with a 2-tailed, unpaired Student t test or compared by 1-way ANOVA followed by the t-test and are expressed as mean ± SD. A value of \( P \leq 0.05 \) was considered statistically significant.

Acknowledgements

This study was supported by grants from National Natural Science Foundation of China (81270221 to J.N.W, 81170095 to J.M.T), Hubei University of Medicine Innovative team project (FDFR201601 to J. M.T), and National Institutes of Health (HL093429 and HL107526 to S.Y.C.).

Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2017.11.061.

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