Author’s response to reviews

Title: Shear stress improves the Endothelial Progenitor Cell function via the CXCR7/ERK pathway axis in the Coronary Artery Disease cases

Authors:

Hua Zhou (zhouhua1022@sina.com)
Qiăng Tu (609538829@qq.com)
Yan Zhang (11259662@qq.com)
Hua Qiang Xie (xiehqi@126.com)
Qing Yun Shuai (280540383@qq.com)
Xiao Chuan Huang (1633805294@qq.com)
Jie Fu (m15897856680@163.com)
Zheng Cao (caozheng908@163.com)

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Author’s response to reviews:

Dear Editor
Thank you very much for your decision letter and advice on our manuscript (Manuscript #Shear Stress Improves the Endothelial Progenitor Cell Function via the CXCR7/ERK axis among the Coronary Artery Disease Cases) entitled “BCAR-D-20-00036R3”. We also thank the reviewers for the constructive and positive comments and suggestions. Accordingly, we have revised the manuscript. All amendments are highlighted in red or blue in the revised manuscript. In addition, point-by-point responses to the comments are listed below this letter. This revised manuscript has been edited and proofread by a native English speaker. We hope that the revision is acceptable for the publication in your journal.

Look forward to hearing from you soon.
With best wishes,
Yours sincerely,
Cao Zheng

Technical Comments:
Editor Comments:
A few changes are still needed, as follows:
Please explain every abbreviation before using it.
Response: Thanks. We revised it.

Background, page 9, lines 19-24. You state: “Our work sheds more light on EPCs-related dysfunction in CAD patients offering a new strategy in the prevention and treatment of CAD.”

Please remove this phrase from Background and include it into Discussion.

Response: Thank you for your comments. We revised it.

Table 1: Please explain all used abbreviations in the footnote!

Response: Thanks. We revised it.

Reviewer reports:

Reviewer 2: According to the statistical analysis: "The data were presented as mean±standard deviation (SD). The SPSS 18.0 software was used for statistical analyses. The unpaired Student's t-test (one-tailed distribution) was employed to compare the two groups. The variance test (ANOVA) followed by the post-hoc test was used to evaluate the statistical significance of multiple groups of data with a parametric distribution. A difference of P<0.05 indicated a statistical significance."

The authors used only t-test to compare the groups. That pre-requisites the normal distribution of the data. Did the authors check the distribution of the data with any of the existing statistical tools?

Response: Thanks, we revised the MS accordingly. (The data were presented as mean±standard deviation (SD). The SPSS 18.0 software was used for statistical analyses. The Kolmogorov-Smirnov test was used to study the distribution of the variables. The unpaired Student t-test or Welch's t-test (normal distribution) was employed to compare the two groups. The variance test (ANOVA) followed by the post-hoc test was used to evaluate the statistical significance of multiple groups of data with a parametric distribution. A difference of P<0.05 indicated a statistical significance.)

Reviewer 3: This is an excellent study evaluating the important role of CXCR7 in mediating shear stress induced protective effects in EPC. Data were consistent and convincing to support the original hypothesis. The reviewer has two comments to address: whether disturbed blood flow has the opposite effect on CXCR7 expression and EPC function as opposed to fluid shear stress described here? What's the mechanism of CAD driven CXCR7 downregulation and shear induced CXCR7 upregulation, any role of mechanosensitive transcription factor KLF2? The author can use data or discuss both comments. There are some typos in title.

Reply: Thank you for your comments.

Previous studies have shown that the disturbed blood flow is related to vascular diseases, including atherosclerosis, restenosis, and arteriosclerosis[1]. The lifespan of endothelial cells in the arterial wall is limited, e.g., 12 months in the areas with the laminar flow, but cells in the regions with the disturbed blood flow may live only weeks[2]. Recent study by Foteinos et al. demonstrated that the numbers of death/proliferating cells on the aortas of ApoE-/- mice were significantly different to wild-type animals. Importantly, lesion-prone areas display a higher turnover rate of endothelial cells as indicated by BrdU positive staining. Therefore, endothelial cells in these affected areas undergo a higher death/proliferation cycle in the presence of hyperlipidaemia and disturbed blood flow[3]. Zeng et al. found that the disturbed flow can also result in endothelial apoptosis, which is mediated by endoplasmic reticulum stress response via the activation of X-box binding protein 1(XBP1)[4]. Based on these studies, we assumed that the
disturbed blood flow could impair the function of EPCs and even cause cell death. However, whether CXCR7 is involved in disturbed blood flow-related decline of EPCs function is still unknown.

Kruppel-like factor 2 (KLF2), a member of the zinc finger transcription factor family, regulates cellular growth during tissue development[5]. Studies have demonstrated that KLF2, which may be induced by shear stress, contributes to maintaining a healthier endothelium by inducing a quiescent state, in addition to having antithrombotic, vasorelaxing and anti-inflammatory effects[6]. And one previous study demonstrated that shear stress may induce differentiation of EPCs to ECs in a magnitude-dependent manner through its effects on the integrin-actin cytoskeleton system, and may result in the increased expression of von Willebrand factor (vWF) and cluster of differentiation 31 (CD31) under a shear stress of 12 dyn/cm²[7,8]. Hai et al. demonstrated that shear stress could upregulate KLF2 expression, while blocking integrin β1/β3 or destroying F-actin resulted in a corresponding decrease in KLF2 expression. Downregulation of KLF2 expression by siKLF2 inhibited the differentiation of EPCs to ECs under shear stress conditions, while the expression of EC-specific markers decreased, including CD31 and vWF[9]. So shear stress could promote the EPCs vasculogenic function through KLF2 signaling. But, the relationship between KLF2 and CXCR7 has not been elucidated yet, and further studies are required to investigate the functional effects. Indeed, our study indicated that the abnormal CXCR7 signaling in EPCs participated in the impaired CAD-derived EPCs function, but the exact mechanism responsible for diminished CXCR7 expression remains to be further investigated. Our next work will focus on the mechanism of CAD driven CXCR7 downregulation and shear induced CXCR7 upregulation. Thanks.