Author’s response to reviews

Title: Abnormal phosphorylation of Tie2/Akt/eNOS signaling pathway and decreased number or function of circulating endothelial progenitor cells in prehypertensive premenopausal women with diabetes mellitus

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Reviewer #2:

1. The first observation related to the presented work is the lack of control patients normotensive, premenopausal and diabetic. The authors demonstrated that the number and function of circulating EPCs is altered in prehypertensive, premenopausal and diabetic women. Since prehypertensive (non-diabetic) women had no alteration the question is: Is this alteration associated with the diabetes? Normotensive, premenopausal and diabetic women could be a control group able to answer this question.

Response: Thank you for your valuable suggestion. As you suggested, we have recently added twenty normotensive premenopausal women with diabetes mellitus as a control group. These added data were shown in Tab1 and Figure1-4. When compared with normotensive or prehypertensive premenopausal women without diabetes mellitus, the number or function of circulating EPCs and flow-mediated dilatation (FMD) in normotensive or prehypertensive premenopausal women with diabetes mellitus decreased. In parallel, the plasma NO level or NO secretion of circulating EPCs in normotensive or prehypertensive premenopausal women with diabetes mellitus was also reduced. These results indicated that in presence of diabetes mellitus, the number and function of circulating EPCs is decreased in both normotension and prehypertension premenopausal women.

2. In relation to the role of diabetes in number and function of circulating EPCs, the authors mentioned in the text that it is still known that the disease induces a reduction in these cells
and their function. So it is clear that those alterations are direct causes of diabetes and are not related to prehypertensive status. Since authors showed that there are no alteration in function of circulating EPCs between hypertensive and normotensive women (no diabetic), why is the new knowledge covered by this work?

Response: Thank you for your valuable suggestion. Our previous studies proved that the number and activity of circulating EPCs in prehypertensive premenopausal women were preserved [1]. However, whether the beneficial effect still exists in prehypertensive premenopausal women with diabetes mellitus is still not clear. The present study further demonstrates that diabetes can abolish the preserved number and activity of circulating EPCs in premenopausal prehypertensive women, indicating that it may be necessary to improve vascular repair capacity for prehypertensive premenopausal women with diabetes mellitus.

Reference:


3. Why the proliferation assay was done with cells in absence of serum but measurement of NO, VEGF and GM-CSF was quantified in supernatant of cell growing in serum 20%? It is known that serum is able to modified growth rates of cells and the secretion of growth factors.

Response: Thank you for your valuable suggestion. The measurement of NO, VEGF and GM-CSF in our manuscript was performed according to the previous study and our published methods [1, 2]. Although the serum may contain some growth factors, the concentration of in serum in cell culture medium is identical and the EPC culture condition is similar. Therefore, it may have little effect on the measurement of NO, VEGF and GM-CSF in the conditioned media.

Reference:


4. The item "Measurement of Plasma NO, VEGF and GM-CSF levels" starts describing the measurement of these molecules in cell culture supernatants. Is it right? The title of the item mentioned measurement in plasma. Additionally, the item "Measurement of NO, VEGF and GM-CSF secretion by EPCs" seems describe the NO assay again but do not describe how the other molecules (VEGF and GM-CSF) were quantified (ELISA?)

Response: We agree with you. As you suggested, we have corrected these mistakes in the revised manuscript.

5. The legend of figure 1 needs revision. In the first phrase there are repetition of words like "evaluated" and "by".

Response: Thank you for your thoughtful view. we have corrected it in the revised manuscript.

6. Maybe the molecular results about Tie2/Akt/eNOS are the great news of the work. However, the authors only show a cause-consequence of diabetes in NO production and circulating EPCs function. It would be interesting if the authors try something to prove this association at the molecular level maybe working with siRNA.

Response: Thank you for your valuable suggestion. We will further clarify the association between Tie2/Akt/eNOS signal pathway and circulating EPCs function at the molecular level in the future investigation.