Author's response to reviews

Title: Age-dependent defective TGF-beta1 signaling in patients undergoing coronary artery bypass grafting.

Authors:

Santiago Redondo (santiredondo@hotmail.com)
Jorge Navarro-Dorado (jorgenavdor@hotmail.com)
Marta Ramajo (mramajo@bio.ucm.es)
Ursula Medina (mmuf_18@hotmail.com)
Pedro Molina-Sanchez (pmolina@cnic.es)
Zaady Garces (zaqazul@hotmail.com)
Mauricio Alonso (cirgad2.hcsc@salud.madrid.org)
Fernando Reguillo (fjrl1965@yahoo.es)
Enrique Rodriguez (circar.hcsc@salud.madrid.org)
Vicente Andres (vandres@cnic.es)
Teresa Tejerina (teje@med.ucm.es)

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Author's response to reviews: see over
Major comment

Molecular mechanisms whereby aging is associated with lower degrees of TGFbeta1 production and p27 expression remain unexplored. This reviewer proposes authors to perform at least following two sets of experiments.

1) protein/mRNA analysis within the VSMC culture.

Using VSMC cultures derived from aging patients, authors can perform immunoblot/qRT-PCR to analyze TGFbeta1 transcript expression within the cells. This will allow the authors to dissect at which level TGFbeta1 production-secretion is perturbed in aged men-derived VSMC.

As stated by the Reviewer, TGF-beta1 is regulated also by transcription. However, it is recognized that it suffers post-transcriptional regulations, as well as a tightly regulated intracellular trafficking, secretion and activation from the binding peptide. This issue has been commented in the text, as suggested (page 12, lines 16 and 17).

2) Smad regulation.

The authors focused on p27 as marker of TGFbeta1 signal in their IMA experiments. p27 can be also regulated, however, by a number of other cellular stimuli. The principal signal transducing module of TGFbeta1
receptor system are the Smad proteins, which undergo phosphorylation at specific residues when the receptors are liganded by the TGFbeta1. I would therefore propose that the authors reprobe membranes of IMA experiments (for example the one in the Fig 2A) for phosphorylated as well as total of Smad2/3 proteins.

This new set of experiments has been performed in the current version of the manuscript, as suggested. However, no significant differences were found (current Figure 3).
REVIEWER 2.

Major Compulsory Revisions: None

Minor Essential Revisions:

In the abstract, it is not clear which patients (CABG or abdominal patients) provide which sample (arteries, plasma,...). The reader has to go to the Methods and Result section to get that information. Please rephrase the abstract to clarify. How many abdominal surgery patients?

This issue has been clarified in the current version of the manuscript, as suggested (page 2, lines 9-11).

Is there a relationship between TGF-b levels and pharmacological treatment (ie, do statins/ACEI/betablockers affect TGF-b signaling) in this population?

No significant differences were noted (page 9, lines 15-17).

Discretionary Revisions (recommendations for improvement which the author can choose to ignore):

Is there any relationship between TGF-# levels and severity of atherosclerosis (eg, % of maximal stenosis in coronariography, number of
coronary segments affected, Framingham score, Agatston score,..., or rough estimates such as LVEF or wall motion score index or positivity of conventional stress tests -echo, nuclear-) even after adjusting by age? This result would greatly enhance the message conveyed by the authors.

As stated by the Reviewer, this information may enhance our message. However, the vast majority of our patients underwent surgical CABG given that they had coronary disease in the three major vessels. Unfortunately, in many cases only this information was available and this is a limitation of the current manuscript. Deeper study about the charge of cardiovascular disease will be the subject of further reviews. This issue has been discussed in the current version of the manuscript, as suggested (page 13, lines 10-12).

Since TGF-b acts mainly through the SMAD pathway, the finding of age-dependent SMAD signalling pathway in the internal mammary arteries would also reinforce the results.

This new set of experiments has been performed in the current version of the manuscript, as suggested. However, no significant differences were found (current Figure 3).