TSC-22 regulates collagen 3a1 gene expression in the rat heart

In their paper, Kelloniemi and co-authors studied the role of the transcription factor TSC-22 in cardiac remodeling. The authors reported that TSC-22 gene expression is up-regulated in left ventricular tissue by multiple hypertrophic stimuli such as angiotensin, AVP, and endothelin, as well as after myocardial infarction. In contrast, treatment of rats with both the AT1-receptor antagonist losartan and the beta-blocker metoprolol significantly reduced TSC-22 gene expression levels in the heart. In cultured neonatal ventricular myocytes, stimulation with endothelin-1 resulted in increased TSC-22 expression both at protein and mRNA level. In addition, the authors studied the effects of adenovirus-mediated TSC-22 gene transfer in normal rat hearts. Localization of the transgene was assessed by means of immunohistochemical staining, and gene expression in the myocardium was studied using RT-PCR. The authors demonstrated enhanced expression of collagen type IIIalpha1 in left ventricular tissue upon adenovirus-mediated delivery of the TSC-22 transgene.

The paper is well-written and the authors used a broad range of experimental approaches to investigate the role of the leucine zipper protein TSC-22 in cardiac remodeling and fibrosis, including in vitro and in vivo studies. However, this reviewer is concerned about the generally poor quality of the Western blot data, given the weak signal intensity and the fact that usually not more than only one band per treatment group is shown to the reader (see Figures 1A, 4E, and 6B). Furthermore, loading controls were not performed. To me, it appears as if some of the blots shown in the figures were reassembled from different gel runs. Here, the authors should be advised to present their original blots. This issue is of importance because the conclusions drawn from the experiments are mainly based on immunoblotting (and RT-PCR) data. Similarly, the original Northern blot in Figure 2A is missing.

Minor changes:

The title should be more precise indicating that TSC-22 expression up-regulates (and not only modulates) collagen type IIIalpha1 expression in the heart.

Line 44: One sentence on page 17 (“We also observed that growth-promoting factors...”) should be rephrased, because only one factor, namely endothelin-1, was tested for its effect on TSC-22 expression in cultured neonatal rat ventricular myocytes.
myocytes.

Line 45: TSC-22 does not have a consensus DNA-binding domain. However, this does not exclude that the protein binds to specific sequences via a DNA-binding surface analogous but differed from that of other basic leucine zipper proteins (see also Dobens et al., Mechanisms of Development, 1997;65:197). Please be more precise.

Line 54: The abbreviation should be EGF (and not EGF2).

Lines 105/106: Please delete the phrase: “with free access to tap…”

Line 117: Please add the full-length term for the abbreviation AVP: arginine vasopressin

Line 132: Please include the rat strain used in the LAD ligation experiments (see also line 275).

Line 309: losartan-treated SHRs (please add hyphen)

Line 308: There is no Figure 3C.

Line 323: Neither … nor

Line 325: p38alpha/gamma (please use Greek letters)

Line 364: “TSC-22 is proposed to …”

Line 396: AngII-induced (please add hyphen)

Line 398/399: Please rephrase: “However, in transgenic mice overexpressing TGF-beta1…”

Lines 397-399: beta-adrenoceptor (please use Greek letters)

Line 399: AT1-receptor antagonist

Line 440: Please add comma after the reference [53],

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.