

Letter to the editor

Thioredoxin-related protein-1 induced by prostaglandin E₂

Antonio Miranda-Vizuet

Centro Andaluz de Biología del Desarrollo (CABD-CSIC)
Depto. de Fisiología, Anatomía y Biología Celular
Universidad Pablo de Olavide,
41013 Sevilla, Spain

Phone: +34 954 349381

Fax: +34 954 349376

Email: amirviz@upo.es

Dear Sir,

After a careful reading of the paper by Kim *et al*¹ where they report on a thioredoxin-related protein-1 (TRP-1) induced by prostaglandin E₂, I felt compelled to bring to your attention several concerns on the results and conclusions raised in this work.

Although the authors state three times throughout their paper that TRP-1 is a variant of the previously described TMX-2/CGI-31 protein² they do not provide any DNA or protein sequence comparison to illustrate the degree of homology between both variants. When attempting to perform this comparison myself I found that TRP-1 DNA or protein sequences are not available at GenBank and two email requests to the corresponding author of the paper resulted in no reply. Therefore, the only alternative available was to type myself the complete mRNA sequence as published in the printed version and the resulting comparisons are shown in Figure 1 at the mRNA level (95.1% identity) and in Figure 2 at the protein level (92.2% identity). The TRP-1 and TMX-2/CGI-31 mRNAs mainly differ at two different regions centered at nucleotides 552 and 840 (Figure 1, based on TMX-2/CGI-31 nucleotide numbers) both within the ORF. The most striking result of these nucleotide changes is the conversion of the protein sequence WSNDC to WCGPC, which is the canonical sequence of thioredoxins active site³. This feature prompted the authors to name thioredoxin-related protein-1 (TRP-1) to the new variant.

However, the existence of this variant is not sustained either by the available databases or experimental data provided. **First**, there are none EST sequences that code for the TRP-1 variant. **Second**, a detailed inspection of the human genomic databases does not identify any

sequence that would give rise to this variant by alternative splicing. **Third**, the authors use the complete TRP-1 cDNA as probe for Northern blots to identify a 1.7 kb band induced by PGE₂ treatment. This size is identical to that reported previously for TMX-2/CGI-31² and, given the high homology between the two mRNAs, one cannot conclude from this approach that the 1.7 kb band corresponds to TRP-1 and not to TMX-2/CGI-31. Similarly, as the same probe is used to determine the differential expression of TRP-1 mRNA in normal versus cancer tissues, again it is not possible to conclude that the difference pertains to TRP-1 and not to TMX-2/CGI-31. **Fourth**, the authors use real-time quantitative PCR to corroborate the PGE₂ induction of TRP-1 mRNA shown by northern blot. However, the primers used for this approach are centered at position 89 (forward primer) and 429 (reverse primer) and their sequences at these positions are identical for both TRP-1 and TMX-2/CGI-31 variants. Therefore, real-time PCR using the primers described by Kim *et al.* cannot discriminate which variant is induced by PGE₂ treatment.

Taken together, I conclude that there is no physical evidence of the TRP-1 variant being coded by the human genome and expressed in the experimental set up described by Kim *et al.* Thus, a plausible explanation for the isolation of TRP-1 might be as an aberrant product derived from the treatment, the technique or the cell lines used in this work. An irrefutable demonstration of the existence of TRP-1 would come from real-time PCR using specific primers unique for TRP-1 and designed at the two regions previously mentioned that differ from TMX-2/CGI-31 sequence.

Yours sincerely,

A. MIRANDA-VIZUETE

References

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2. Meng X, Zhang C, Chen J, Peng S, Cao Y, Ying K, Xie Y, Mao Y. Cloning and identification of a novel cDNA coding thioredoxin-related transmembrane protein 2. *Biochem Genet* 2003;41:99-106.
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Figure Legends

Figure 1. Comparison of TMX-2/CGI-31 and TRP-1 mRNA sequences. The TRP-1 nucleotides that differ from those of TMX-2/CGI-31 are boxed. The ORF is shadowed and the primers used for real-time PCR are indicated by arrows.

Figure 2. Comparison of TMX-2/CGI-31 and TRP-1 protein sequences. The TRP-1 residues that differ from those of TMX-2/CGI-31 are boxed. The thioredoxin active site of TRP-1 is shadowed.