Comments

TRPC6 in podocytes: questions and commentary on the article by Jiang *et al*., 'Over-expressing transient receptor potential cation channel 6 in podocytes induces cytoskeleton rearrangement through increases of intracellular Ca^{2+} and RhoA activation'

Jiang *et al*.[1] report that over-expression of TRPC6 in cultured podocytes causes rearrangement of the actin cytoskeleton along with a reduction of synaptopodin and nephrin expression and induction of RhoA activation. In light of previous data on calcineurin activation through TRPC6 in cardiac myocytes[2] and undifferentiated conditionally immortalized podocytes[3] as well as calcineurin-dependent cathepsin-L-mediated degradation of synaptopodin,[4] the hypothesis that TRPC6 over-expression may exert at least some of its nocuous effects on podocytes via a loss of synaptopodin is certainly tempting. However, the data presented by Jiang *et al*. contain several serious inconsistencies and therefore do not allow any conclusions to be drawn. We would like to point out in the following just some of the most severe flaws of this study.

(1) Using conventional approaches, differentiated or differentiating cultured podocytes are notoriously difficult to transfect and a transfection efficiency exceeding 5% cannot usually be achieved. While this may be sufficient for morphological analysis of cells transfected with a fluorescently labeled fusion protein, the low transfection efficiency precludes meaningful analysis of cell lysates by Western blot. Such studies would require lentiviral gene transfer or the generation of stable cell lines over-expressing the protein of interest. Jiang *et al*.[1] claim to over-express EGFP-tagged TRPC6 in differentiated podocytes by using Lipofectamine 2000. However, in their Western blot analysis, transfected cells simply show a stronger TRPC6 band at the exact same size as the untransfected or empty-vector transfected cells. An EGFP tag considerably adds to the molecular weight of a fusion protein; hence, Western blot analysis of efficiently transfected cells should reveal a double band: one for endogenous TRPC6 and another for the roughly 27 kDa larger over-expressed EGFP-TRPC6-fusion construct. Thus, whatever the mentioned Western blot by Jiang *et al*.[1] shows, it is not the claimed over-expression of EGFP-TRPC6, but either an unspecific effect or unequal protein loading.

(2) To demonstrate a reduction in synaptopodin protein expression, Jiang *et al*.[1] show a Western blot band of 44 kDa size. Although synaptopodin was originally named pp44 due to an apparent size of 44 kDa on initial Western blots, intact full length synaptopodin long (the podocyte isoform) has an apparent molecular weight of ca. 110 kDa.[5] In fact, the 44 kDa band may represent a cleavage product arising by cathepsin-L-mediated cleavage of synaptopodin.[4]

(3) Instead of using one of the (unspecific) TRPC6 antagonists, such as 2-APB or SK&F 96365, Jiang *et al*.[1] use U73122 as a 'TRPC6 inhibitor', stating that this phospholipase C (PLC) inhibitor inhibits endogenous diacylglycerol (DAG) production and thus abolishes DAG-dependent TRPC6 activation. They show Ca-imaging data claiming that TRPC6 over-expressing cells exhibit an increased response to the DAG analog 1-oleoyl-acetyl-*sn*-glycerol (OAG), which was completely blunted by U73122. However, as OAG acts downstream of PLC, U73122 should not have any effect on OAG-induced TRPC6 activation. The significance of this experiment is therefore highly doubtful.

(4) As a main morphological characteristic of TRPC6 over-expressing podocytes, Jiang *et al*.[1] report retraction of cell processes. However, the massively elongated cell processes shown for wild-type podocytes in this report are not a typical feature of differentiated podocytes *in vitro* and may even be a sign of cellular stress, such as seen with mycoplasma infection.

Taken together, these inconsistencies raise serious concerns about the validity of the data reported by Jiang *et al*.

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REFERENCES

1 Jiang L, Ding J, Tsai H, Li L, Feng Q, Miao J, Fan Q. Over-expressing transient receptor potential cation channel 6 in podocytes induces cytoskeleton rearrangement through increases of intracellular Ca^{2+} and RhoA activation. *Exp Biol Med (Maywood)* 2011;**236**:184–93
2 Kuwahara K, Wang Y, McAnally J, Richardson JA, Bassel-Duby R, Hill JA, Olson EN. TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. *J Clin Invest* 2006;**116**:3114–26
3 Schlondorff J, Del Camino D, Carrasquillo R, Lacey V, Pollak MR. TRPC6 mutations associated with focal segmental glomerulosclerosis cause constitutive activation of NFAT-dependent transcription. *Am J Physiol Cell Physiol* 2009;**296**:C558–69
4 Faul C, Donnelly M, Merscher-Gomez S, Chang YH, Franz S, Delfgaauw J, Chang JM, Choi HY, Campbell KN, Kim K, Reiser J, Mundel P. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat Med* 2008;**14**:931–8
5 Asanuma K, Kim K, Oh J, Giardino L, Chabanis S, Faul C, Reiser J, Mundel P. Synaptopodin regulates the actin-bundling activity of alpha-actinin in an isoform-specific manner. *J Clin Invest* 2005;**115**:1188–98