

REFERENCES

- 1 Okamura M, Takano Y, Hiramatsu N, Hayakawa K, Yao J, Paton AW, Paton JC, Kitamura M. Suppression of cytokine responses by indomethacin in podocytes: a mechanism through induction of unfolded protein response. *Am J Physiol Renal Physiol* 2008;**295**: F1495–503
- 2 Fan Q, Zhang H, Ding J, Liu S, Miao J, Xing Y, Yu Z, Guan N. R168H and V165X mutant podocin might induce different degrees of podocyte injury via different molecular mechanisms. *Genes Cells* 2009;**14**:1079–90
- 3 He FF, Zhang C, Chen S, Deng BQ, Wang H, Shao N, Tian XJ, Fang Z, Sun XF, Liu JS, Zhu ZH, Meng XF. Role of CD2-associated protein in albumin overload-induced apoptosis in podocytes. *Cell Biol Int* 2011;**35**:827–34
- 4 Jia R, Hong X, Li S, Haichun Y, Chuanming H. Expression of angiotensin-like 3 associated with puromycin-induced podocyte damage. *Nephron Exp Nephrol* 2010;**115**:e38–45
- 5 Bleasdale JE, Thakur NR, Gremban RS, Bundy GL, Fitzpatrick FA, Smith RJ, Bunting S. Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. *J Pharmacol Exp Ther* 1990;**255**: 756–68
- 6 Smith RJ, Sam LM, Justen JM, Bundy GL, Bala GA, Bleasdale JE. Receptor-coupled signal transduction in human polymorphonuclear neutrophils: effects of a novel inhibitor of phospholipase C-dependent processes on cell responsiveness. *J Pharmacol Exp Ther* 1990;**253**: 688–97
- 7 Thompson AK, Mostafapour SP, Denlinger LC, Bleasdale JE, Fisher SK. The aminosteroid U-73122 inhibits muscarinic receptor sequestration and phosphoinositide hydrolysis in SK-N-SH neuroblastoma cells. *J Biol Chem* 1991;**266**:23856–62
- 8 Dryer SE, Reiser J. TRPC6 channels and their binding partners in podocytes: role in glomerular filtration and pathophysiology. *Am J Physiol Renal Physiol* 2010;**299**:F689–701
- 9 Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* 1999;**397**:259–26
- 10 Inoue R, Okada T, Onoue H, Hara Y, Shimizu S, Naitoh S, Ito Y, Mori Y. The transient receptor potential protein homologue TRP6 is the essential component of vascular α 1-adrenoceptor activated Ca^{2+} -permeable cation channel. *Circ Res* 2001;**88**:325–32
- 11 Estacion M, Sinkins WG, Jones SW, Applegate MA, Schilling WP. Human TRPC6 expressed in HEK 293 cells forms non-selective cation channels with limited Ca^{2+} permeability. *J Physiol* 2006; **572**:359–77
- 12 Ju M, Shi J, Saleh SN, Albert AP, Large WA. Ins(1,4,5)P₃ interacts with PIP₂ to regulate activation of TRPC6/C7 channels by diacylglycerol in native vascular myocytes. *J Physiol* 2010;**588**:1419–33
- 13 Saleh SN, Albert AP, Peppiatt CM, Large WA. Angiotensin II activates two cation conductances with distinct TRPC1 and TRPC6 channel properties in rabbit mesenteric artery myocytes. *J Physiol* 2006;**577**:479–95
- 14 Mundel P, Reiser J, Zúñiga Mejía Borja A, Pavenstädt H, Davidson GR, Kriz W, Zeller R. Rearrangements of the cytoskeleton and cell contacts induce process formation during differentiation of conditionally immortalized mouse podocyte cell lines. *Exp Cell Res* 1997;**236**: 248–58

Additional comments on response of Jiang *et al.*

We appreciate the response to our concerns by Jiang *et al.* Some issues, however, were addressed inadequately or not at all.

(1) The authors have now provided a Western blot showing a 133 kDa band for EGFR-TRPC6. However, the strong bands at about 106 representing endogenous TRPC6 that are evident in the published paper are now hardly visible in the newly provided blot.

(2) The newly provided, uncropped blot for synaptopodin shows much reduced intensity of the 110 kDa band in the eGFP-transfected cells and a stronger band in the TRPC6-transfected cells. These data argue against the conclusion that synaptopodin expression is reduced by TRPC6. Furthermore, the uncropped Western blot shows no band at 44 kDa which is in contrast to the originally published data, where very strong bands at 44 kDa are shown in several figures.

(3) In contradiction to statements by Jiang *et al.* in their response, none of the cited references 10–14 used U73122 to inhibit OAG-induced TRPC6 activation. These studies used U73122 to inhibit Ca^{2+} responses to histamine or angiotensin II, which act upstream of PLC. Although the study by Estacion *et al.* suggested a potential direct effect of U73122 on TRPC6, the study by Ju *et al.* found that ‘U73122-induced inhibition of anti-PIP₂ antibody-evoked TRPC6/C7 channel activity was overcome by co-application of 10 μM OAG, which rules out the possibility that U73122 was acting as a direct channel blocker’.

(4) Although cultured, differentiated, conditionally immortalized podocytes were described as arborized, process-bearing cells, these do not usually display such extremely enlarged processes as shown in the publication by Jiang *et al.* The significance of such morphological characteristics remains to be determined.

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