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Tunneling nanotube (TNT) formation is independent of p53 expression

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Dear Editor,

Tunneling nanotubes (TNTs) are thin cell-to-cell connected membranous tubes first described in rat pheochromocytoma (PC12) cells.[1] Subsequent studies demonstrated that TNTs are present in a broad range of cell types including primary cells and cancer cell lines. The recent discovery that TNT-like structures exist *in vivo* suggests they may have an important role during tissue development and maintenance.[2,3] TNTs facilitate intercellular transfer of various cellular components and are thought to represent a novel form of cellular communication.[1–3] Recently, it was reported that TNT formation in primary rat hippocampal astrocytes and neurons was dependent on the activation of tumor suppressor protein p53 through cellular stress induction such as hydrogen peroxide (H_2O_2).[4] Considering the broad effect of p53 on numerous cellular functions,[5] we here investigated whether p53 is a key protein needed for TNT formation. We first examined the effect of p53 activation on TNT formation in PC12 and OCI-AML3 (acute myeloid leukemia) cells that express wild-type p53 (p53WT). Both cell lines formed typical TNTs,[1] containing F-actin, but no microtubules and had no contact with the substratum (data not shown). Immunoblot analysis showed basal expression of p53 in PC12 and OCI-AML3 cells and increased p53 expression and activity of the p53 target genes *MDM2* and *p21* upon H_2O_2 exposure (Supplementary Figure S1a, i). When TNT numbers were quantified in cultures grown in the absence or presence of H_2O_2, we found no change in number for PC12 cells and a significant reduction for OCI-AML3 cells (Supplementary Figure S1a, ii). This demonstrated that p53 activation did not induce TNT formation in these p53WT cells.

We then investigated whether p53-negative cells form TNTs, using the p53-null human osteosarcoma cell line SAOS-2. Confocal microscopy clearly showed that SAOS-2 cells also display typical TNTs containing F-actin but no microtubules (Supplementary Figure S1b, i). Statistical analyses revealed 12 TNTs per 100 SAOS-2 cells and no inductive effect of H_2O_2 exposure on TNT formation (Supplementary Figure S1b, ii). We next isolated primary mesenchymal stromal stem cells (MSCs) from bone marrow of a double knock-out (dKO) ($p53^{-/-}$ and mouse double minute 2 ($MDM2)^{-/-}$) C57BL/6 mouse.[6,7] The dKO-MSCs also formed typical TNTs containing F-actin and no microtubules (Supplementary Figure S1b, i). Moreover, H_2O_2 exposure increased the TNT numbers from 18 (control) to 34 TNTs per 100 cells (Supplementary Figure S1b, ii). This clearly demonstrated that p53 is dispensable for TNT formation in SAOS-2 cells and dKO-MSCs.

To study if exogenous expression of p53 was able to induce TNTs on a p53-null background, we co-transfected SAOS-2 cells with p53WT, GFP- or mock-expressing plasmids and a 13x-p53-promoter-GFP reporter plasmid as a marker of p53 expression. No GFP expression was observed in cells transfected with the 13x-p53-promoter-GFP reporter alone or in combination with mock plasmid. However, GFP expression was detected upon co-expression with p53WT (data not shown). When TNTs connected with GFP-positive cells were quantified, we found 38 TNTs per 100 p53-positive cells but no difference in TNT number upon H_2O_2 treatment or in GFP-transfected cells (Supplementary Figure S1c). This indicated that p53 expression in SAOS-2 cells did not promote TNT formation.

In conclusion, the present study demonstrates that TNTs form independently of p53 and the effect of H_2O_2 on TNT formation is cell-type dependent and p53 independent. In support of this, several cell lines are reported to form TNTs express mutated or inactive p53.[2,3] Thus, substantial evidence suggests that p53 is not a master protein for TNT formation and further investigation is needed to dissect the molecular mechanisms underlying their formation.

Conflict of Interest

The authors declare no conflict of interest.

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