Virology 441 (2013) 197–199

Contents lists available at SciVerse ScienceDirect

Virology

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Corrigendum

Corrigendum to "The polyomavirus BK agnoprotein co-localizes with lipid droplets" [Virology 399 (2) (2010) 322–331]

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In our publication "*The polyomavirus BK agnoprotein co-localizes with lipid droplets*" (Unterstab et al., 2010 Virology 399:322–31), we reported that the BKV agnoprotein co-localizes with lipid droplets (LD), but not with the endoplasmic reticulum (ER) (Unterstab et al., 2010). In that study, we detected transiently expressed His-tagged BKV agnoprotein (agno-His) in Vero cells using a monoclonal His-tag antibody (DIA900, Dianova, Hamburg, Germany) together with a rabbit antiserum against the ER marker protein calnexin (ADI-SPA860, Enzo Life Sciences, Lausen, Switzerland). In confocal microscopy analysis, we were unable to detect significant overlapping signals of BKV agnoprotein and calnexin (see Supplementary figure 1a in Unterstab et al., 2010).

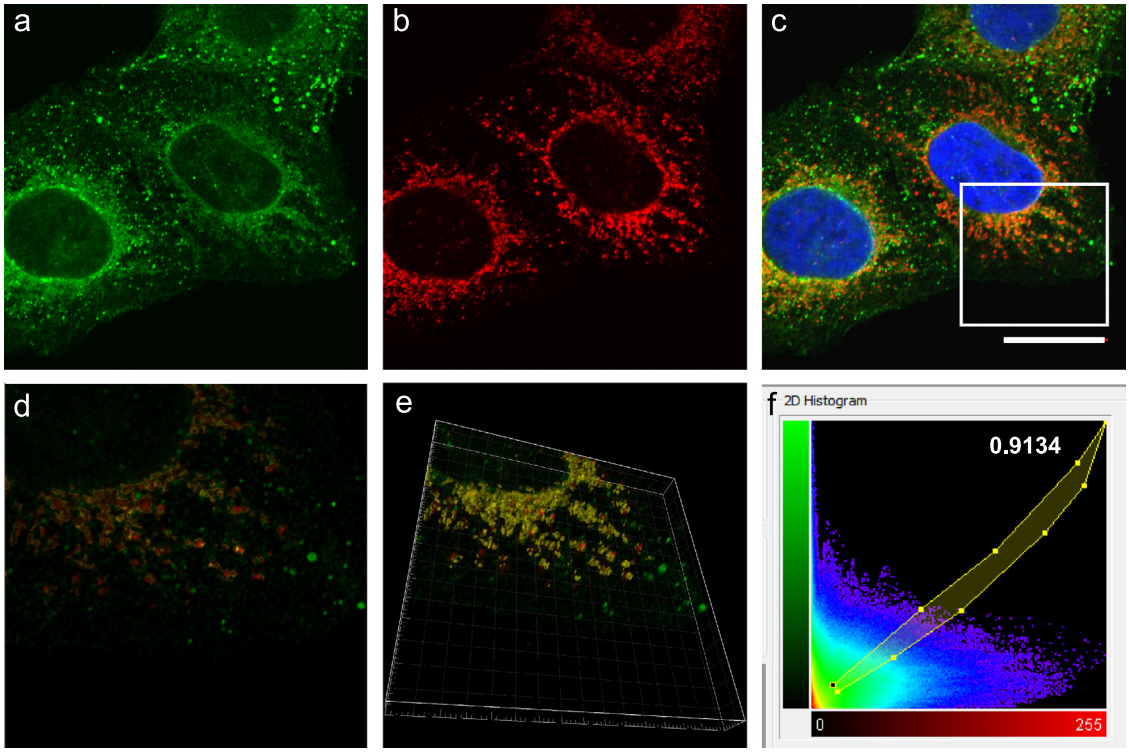

Fig. 1. Co-localization studies for BKV agnoprotein and the ER-localized protein disulfide isomerase (PDI). Immunofluorescence analysis of UTA-6 cells expressing BKV-agnoprotein under a tetracycline-repressible (Tet-off) promoter after fixing the cells with 4% paraformaldehyde (RT, 10 min). (a) Detection of the agnoprotein; (b) PDI; (c) merged channels (Bar 20 μm); (d) Z-slice of a deconvolved 3D image of the indicated area, after co-localization analysis. Co-localizing pixels are shown in a new channel, yellow; (e) volume with isosurface rendering of the co-localization channel; (f) Scatterplot/2D histogram displaying the Pearson coefficient.

DOI of original article: http://dx.doi.org/10.1016/j.virol.2010.01.011
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http://dx.doi.org/10.1016/j.virol.2013.03.018

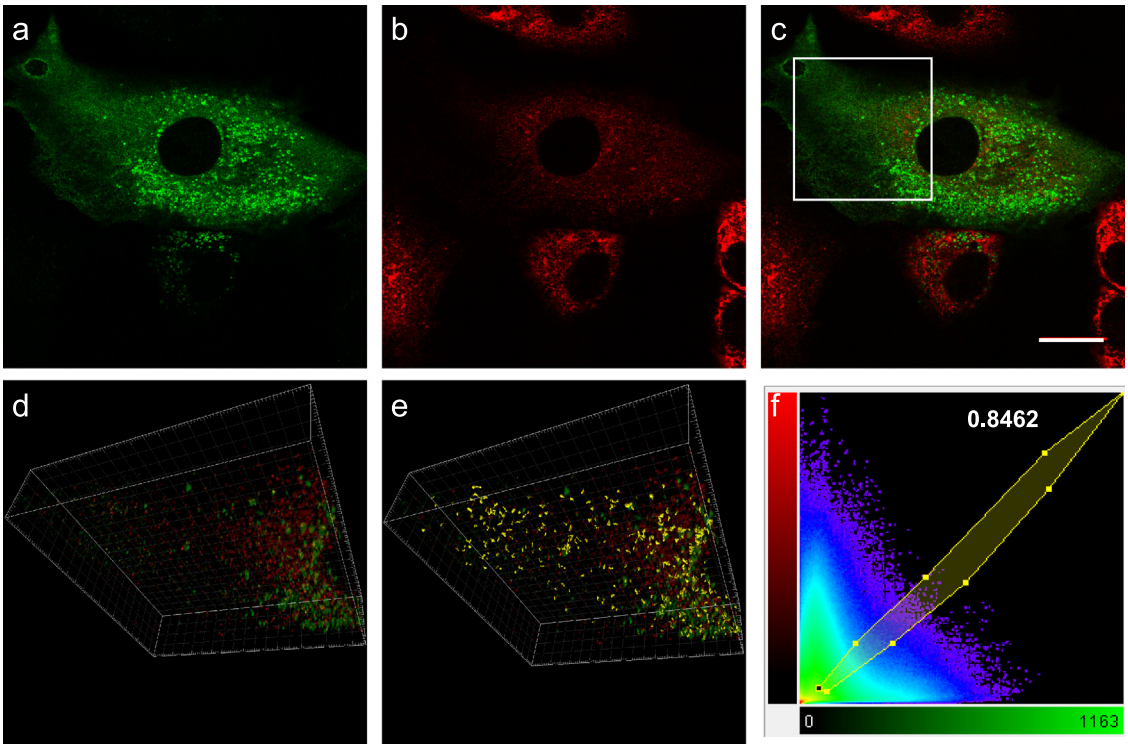

Fig. 2. Co-localization studies for BKV agnoprotein and the ER-localized PDI in BKV Dunlop infected RPTECs at 2 days post infection. (a) Detection of the agnoprotein; (b) PDI; (c) merged channels (bar 20 μm); (d) volume of a deconvolved 3D image of the indicated area, after co-localization analysis. Co-localizing pixels are shown in a new channel, yellow; (e) Volume with isosurface rendering of the co-localization channel, yellow; (f) Scatterplot/2D histogram displaying the Pearson coefficient.

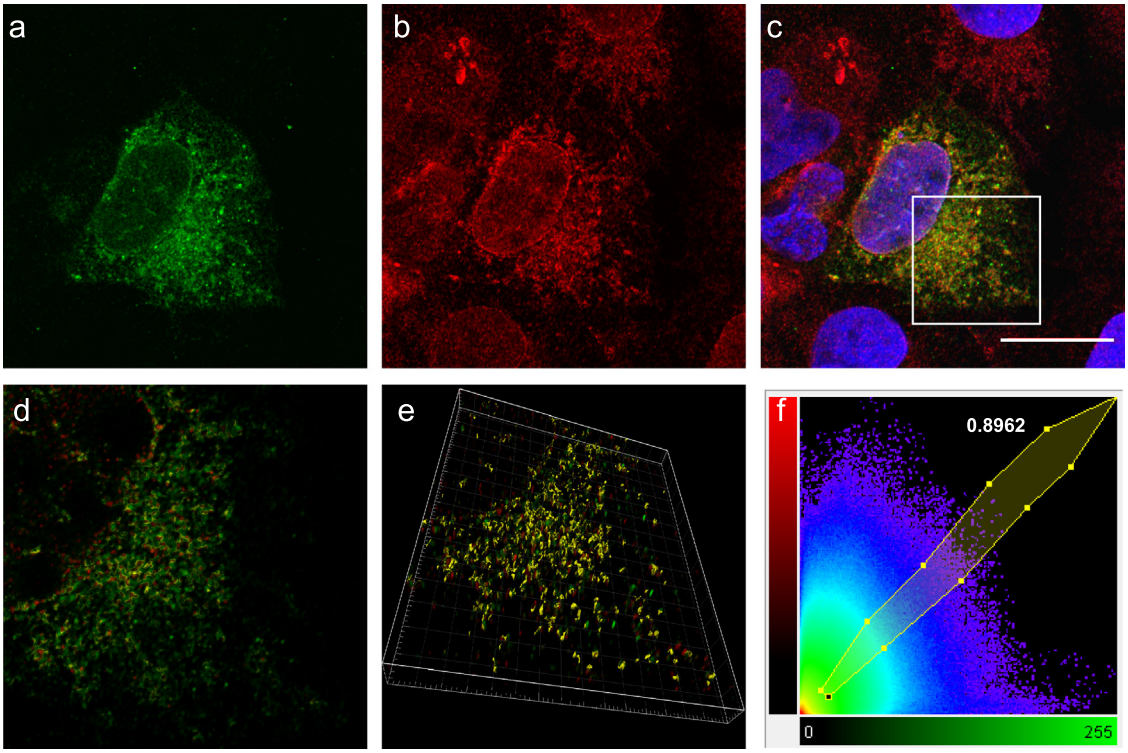

Fig. 3. Co-localization studies for BKV agnoprotein and the ER-localized protein calnexin. Immunofluorescence analysis of UTA-6 cells expressing BKV-agnoprotein under a tetracycline-repressible (Tet-off) promoter after fixing the cells with 4% paraformaldehyde (RT, 10 min). (a) Detection of the agnoprotein; (b) calnexin; (c) merged channels (Bar 20 μm); (d) Z-slice of a deconvolved 3D image of the indicated area, after co-localization analysis. Co-localizing pixels are shown in a new channel, yellow; (e) Volume with isosurface rendering of the co-localization channel, yellow; (f) Scatterplot/2D histogram displaying the Pearson coefficient.

However, further research in our lab led us to reinvestigate the co-localization of BKV agnoprotein. We now found that BKV agnoprotein indeed co-localizes with LD, but also with the ER. This ER co-localization was demonstrated in BKV (Dunlop)-infected primary human proximal tubular epithelial cells (hRPTECs) as well as in UTA-6 cells 2C9 stably expressing Tet-off regulated BKV-agnoprotein at 48 h after tetracycline removal (Cioni et al., in press). BKV agnoprotein was stained together with the ER marker protein protein disulfide isomerase (PDI) using an anti-BKV agnoprotein serum (Rinaldo et al., 1998) and a monoclonal antibody (ADI-SPA-891, 1:250, Enzo Life Sciences, Lausen, Switzerland), respectively. After stack acquisition with 44 z-slices (voxel *xyz* size = 30, 30, 130 nm), de-convolution, and image analysis with Imaris, we clearly detected co-localization of both proteins. Defining a third channel (yellow), which displayed areas of overlapping signals yielded an almost ideal (1.0) Pearson coefficient of 0.9134 (Fig. 1). Similar results were obtained with the ER-marker calreticulin using the chicken polyclonal serum (ab14234, 1:500, abcam, Cambridge, UK; Supplementary Fig. 1). Importantly, BKV agnoprotein co-localization was detected in hRPTECs at 2d post infection with BKV Dunlop (Fig. 2).

To investigate the discrepancy between our previous and present observations, we also re-examined co-localization using a new lot of the anti-calnexin antiserum (ADI-SPA860, 1:200, Enzo Life Sciences, Lausen, Switzerland) described in our original article, for the detection of the ER in UTA-6 cells transiently expressing Flag-tagged BKV agnoprotein (anti-Flag M2 mab, 1:500, Sigma Aldrich, Buchs, Switzerland). This independently demonstrated co-localization of BKV agnoprotein and calnexin (Fig. 3).

The reason for the inconsistency with our previously published data is not clear, but might be attributable to lot-specific issues of the calnexin antiserum and/or to the His-tagged BKV agnoprotein construct. We wish to sincerely apologize for potentially negative effects of our report and hope that our clarification of the BKV agnoprotein co-localization will help to spawn further research resolving the as yet unclear role of BKV agnoprotein.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2013.03.018.

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