OPEN

Citation: Cell Death and Disease (2014) 5, e977; doi:10.1038/cddis.2013.446

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Correspondence

Methodological pitfalls in the study of DAX-1 function

E Lalli*,[1,2,3]

Cell Death and Disease (2014) **5,** e977; doi:10.1038/cddis.2013.446; published online 2 January 2014
Subject Category: Cancer

Dear Editor,

I have read with much interest the recent paper published in *Cell Death and Disease* by Lanzino *et al.*[1] In that paper, the authors propose the existence of a regulatory mechanism used by androgens to repress aromatase expression in breast cancer cells that involves the orphan nuclear receptor DAX-1. I would like to attract the readers' attention on the fact that in that paper all experiments involving visualization of DAX-1 were obtained using the commercial K-17 antibody from Santa Cruz Biotechnology. I believe that extreme caution should be taken to evaluate results generated by the use of that antibody. Helguero *et al.*[2] compared the performance of several antibodies directed against DAX-1 in immunoblot and immunofluorescence. They found that the K-17 anti-DAX-1 antibody recognizes with high affinity a 65-kDa band (DAX-1 molecular weight is 52 kDa) in HeLa whole-cell extracts. This cell line expresses only extremely low levels of DAX-1 mRNA. Moreover, immunofluorescence experiments using the K-17 anti-DAX-1 antibody showed a homogeneous nuclear signal in cell types (HeLa and T47-D) that resulted negative when stained with other anti-DAX-1 antibodies (2ZH7, A0179 and 2F4) that recognized specifically a band of molecular weight around 50 kDa in immunoblot. Those results are entirely consistent with our own data showing that in HeLa cells transfected with a DAX-1 expression vector and costained with the rabbit K-17 and the mouse 2F4[3] anti-DAX-1 antibodies, only a few cells, as expected, are stained with the 2F4 antibody with a prevalently nuclear pattern, whereas all cells are homogeneously stained by the K-17 antibody in the nucleus (Supplementary Figure 1). Taken together with the results previously published by Helguero *et al.*,[2] these data show that the K-17 antibody nonspecifically recognizes a nucler antigen expressed at high levels in HeLa cells and in all other cell lines analyzed (our unpublished observations). Several other studies in addition to Lanzino *et al.*[1] have also used the K-17 antibody to assess DAX-1 expression, both in immunoblot and in immunofluorescence/immunohistochemistry (see Lalli and Alonso[4] for a review), without checking its specificity. The purpose of this correspondence is then to alert scientists working in the DAX-1 field about the pitfalls linked to the use of the K-17 antibody to detect DAX-1 in cell lines and tissues and to encourage the use of more specific antibodies against DAX-1.

Conflict of Interest

The author declares no conflict of interest.

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Supplementary Information accompanies this paper on Cell Death and Disease website (http://www.nature.com/cddis)

[1]Institut de Pharmacologie Moléculaire et Cellulaire CNRS, Valbonne 06560, France; [2]Associated International Laboratory (LIA) NEOGENEX CNRS, Valbonne 06560, France and [3]University of Nice-Sophia-Antipolis, Valbonne 06560, France
*Corresponding author: E Lalli, Institut de Pharmacologie Moléculaire et Cellulaire CNRS, 660 route des Lucioles – Sophia Antipolis, Valbonne 06560, France. Tel: +33 4 93 95 77 55; Fax: +33 4 93 95 77 08; E-mail: ninino@ipmc.cnrs.fr