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Correspondence

Response to Lalli's comment: May the study of DAX-1 function just rely on its visualization?

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If a man will begin with certainties, he shall end in doubt; but if he will be content to begin with doubts, he shall end in certainties.
(Francis Bacon, 1561–1626)

Dear Editor,

In our recent article[1] published by *Cell Death and Disease*, we identified DAX-1 as an androgen-target gene highlighting the existence of a functional androgen receptor/DAX-1/aromatase interplay in estrogen-dependent breast cancer cells. Our conclusion is based on multiple technical approaches. As reported in the article, DAX-1 expression in breast cancer cells in response to androgen treatment has been evaluated not only by western blotting and immunofluorescence assays, but also by mRNA analysis showing a strong increase of DAX-1 expression following androgen administration. Furthermore, the importance of androgens in DAX-1 regulation and function was additionally assessed by DAX-1 promoter studies (luciferase-reporter assays, site-directed mutagenesis studies, DNA affinity precipitation assay, electrophoretic mobility shift assay and chromatin immunoprecipitation assay), and by studies on the effect of DAX-1 silencing or overexpression.

Anyway, we express our sincere appreciation to Lalli[2] for taking the time to lay its concerns out regarding the use of the K-17 Santa Cruz Biotech antibody for DAX-1 visualization. In the very early step of our research study, in hormone-dependent MCF-7 breast cancer cell line, we compared the performance of several DAX-1 antibodies. Also in the study by Helguero *et al.*,[3] only partially cited by Lalli[2] in his letter, authors tested different DAX-1 antibodies, including K-17, by comparing antibodies specificity using the same protein preparations (Figure 1).[3] In this paper, all of the antibodies tested detected DAX-1 in ovarian tissue lysate by western blotting, but none of them detected DAX-1 in DAX-1-negative HeLa or T47-D cells. Nevertheless, these two cell lines were stained with K-17 for immunofluorescence analysis. From the data of these experiments (Figures 1 and 2),[3] the authors conclude that '.....in immunofluorescence studies, K-17 antibody might be cross-reacting with other nuclear epitopes' and dismiss it in this type of analysis. However, the authors did not exclude the use of K-17 for western blotting analyses. In fact, K-17 was used in the study by Helguero *et al.*[3] for further evaluation of DAX-1 in a DAX-1-positive cell line (HC11 mouse mammary epithelial cells) by western blotting analysis (Figure 4).[3] In our experimental system, by using the K-17 antibody, we discriminated the specific DAX-1 band of ~50 kDa MW, from the non-specific one at 60 kDa, as they did in Helguero *et al.*[3] As shown in Supplementary Figure 1, specificity of the ~50-kDa band was proved by DAX-1 silencing experiments. We also want to underline that choosing the K-17 antibody was further supported by the use of this antibody in several other studies.[3–9] We agree with Lalli[2] that in immunofluorescence analysis discrimination between specific and non-specific immunostaining signal is not possible although, in our study, an increase in DAX-1 nuclear signal upon androgen treatment can be seen. Anyway, taking into account this criticism, immunofluorescence studies, aimed to DAX-1 visualization, are reported along with western blotting analysis discriminating the ~50-kDa DAX-1 band.

In conclusion, we would like to emphasize that our original aim was to demonstrate the existence of a novel androgen receptor-mediated mechanism controlling the expression of DAX-1 and consequently of aromatase in a hormone-dependent breast cancer cell line. To accomplish this, we have confidence that precise and consistent methodology has been applied. The title of the Letter to the Editor by Lalli[2] is provocative but, based on the facts mentioned, of questionable scientific validity: does Lalli[2] think that the study of DAX-1 function may just rely on its visualization?

Conflict of Interest

The authors declare no conflict of interest.

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