Author's response to reviews

Title: Hyperglycemia regulates thioredoxin-ROS activity through induction of thioredoxin-interacting protein (TXNIP) in metastatic breast cancer-derived cells MDA-MD-231

Authors:

Francesco Turturro (fturtu@lsuhsc.edu)
Ellen Friday (efrida@lsuhsc.edu)
Tomas Welbourne (twelbo@lsuhsc.edu)

Version: 2 Date: 1 May 2007

Author's response to reviews: see over
May 1, 2007

BioMed Central Editorial

RE: MS1209134653129870-Hyperglycemia regulates thioredoxin-ROS activity through induction of thioredoxin-interacting protein (TXNIP) in metastatic breast cancer-derived cells MDA-MB-231

Dear Editor,

We are grateful that we have been given the chance to resubmit our manuscript for consideration of publication in BMC Cancer. We appreciated the comments of the reviewers and we have tried to satisfy them as much as we could.

Please, find attached a point-by-point response to the reviewers’ concerns.

Sincerely,

Francesco Turturro, MD
Corresponding Author

1) Response to Reviewer MG Daidone

We specified that is a two-tailed P-value in the Methods’ section. We also address the necessity of studying the described functional axis in breast cancer-derived cells as related to hormonal status of the cells, signaling pathways etc, which was not the intent in this initial exploratory study. We also corrected MDA-MB-231 cells and use GEP consistently.

2) Response to Reviewer J Yodoi

1. Although the statistical validity of duplicates instead of triplicates has been questioned by Dr. Yodoi, experiments may be repeated 3 or more times, but realistically, choices have been made on available resources (limitations of funding, etc.). The decision to do fewer replicates at different levels is often more logistical than scientific. However, two data points are sufficient to get a standard deviation and
a confidence interval. More replicates could define the estimates but in this case the conclusions of this paper would not be different. The reviewer can refer to a few references on standard deviations and confidence intervals: 1) Intuitive Biostatistics by Harvey Motulsky, President, GraphPad Software Publisher, Oxford University Press, New York, 1995; 2) Biostatistical Analysis, 2nd edition by Jerrold H, Zar Prentice-Hall, 1984, ISBN 0-13-077925-3.

2. This was specified in the Method section as requested.

3. Figure 3A refers to chronic conditions, which means that those cells have been maintained in stable conditions of growth with either 5 or 20 mM glucose. The measurements refer to these conditions that are independent from the time of measurements. We included in Figure 4B the most representative flow cytometry histogram as requested.

4. We did not measure ROS level and TRX activity, because our scope was to demonstrate the relationship between intracellular levels of glucose and regulation of TXNIP levels. This concept is well documented and elaborated in the discussion section. We considered a redundancy showing relationship between TXNIP and ROS/TRX since our intent was not to relate GLUT1 inhibition to this final effect but rather glucose transport to TXNIP levels.

5. Although our work was not meant to investigate the mechanistic relationship between diabetes and cancer, we elaborate more in the discussion on this matter based on our very initial observation.

We highlighted the relevance of the references as suggested, although the JBC 2004 by Schulze was already in our references.

3) Response to reviewer F McCormick

We addressed all the concerns in the discussion of the revised version as requested elaborating on the relevance of these comments. We have certainly addressed the cell-dependence of the TXNIP and breast cancer-derived cells with various malignant phenotype in another work which is in press.