Author's response to reviews

Title: The Effect of Bisphosphonates on gene expression: GAPDH as a housekeeping or a new target gene?

Authors:

Maria Teresa Valenti (valenti.lab.oncnoale@inwind.it)
Francesco Bertoldo (francesco.bertoldo@univr.it)
Luca Dalle Carbonare (luca.dallecarbonare@univr.it)
Giuseppe Azzarello (giuseppe.azzarello@libero.it)
Sonia Zenari (soniazenari@univr.it)
Mirko Zanatta (zanatta@univr.it)
Elena Balducci (e.balducci@libero.it)
Orazio Vinante (orazio.vine@libero.it)
Vincenzo Lo Cascio (vincenzo.locascio@univr.it)

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Author's response to reviews:

To
Peter Newmark
Editor in chief of BMC - Cancer

Dear Professor Newmark,

please find enclosed the revised version of the paper entitled: "The Effect of Bisphosphonates on gene expression: GAPDH as a housekeeping or a new target gene?", submitted for a publication in BMC Cancer.

The corrections according to the referees' suggestions are reported in italic in the text. In addition, we answered point-by-point to all the reviewer's queries:

Reviewer: Jean-Philippe Peyrat:

. Pag. 3 line 8: we corrected the spelling mistake.
. Pag. 4 line 15: on this basis and since the GAPDH is commonly used as housekeeping gene in gene expression studies also in models using bisphosphonates and since it is up-regulated in many cancer (7-9,11) and down-regulated by chemotherapics (6) we explored the influence, if any, of some bisphosphonates commonly used in cancer bone disease on GAPH gene expression in breast and prostate cancer cell lines.
. Pag. 5: the meaning of ddH2O is double distilled water.
. Pag. 6: the quantity of total RNA was: 1 mg.
. Pag. 6 line 11: the space was added and the meaning of B2M was specified (beta-2 microglobulin).
. Pag. 6 line 12: we corrected the spelling mistake.
. The tables 1 and 2 have been removed.
. Pag. 15 ref7: we corrected the spelling mistake.
. Pag. 20: we corrected the spelling mistake

Reviewer: Rob Coleman:

MAJOR COMPULSORY REVISION:

We utilized standard procedures that assure the drop out of non adherent apoptotic elements and of degraded mRNA deriving from apoptotic cells. We also used the same quantity of ribonucleic acid from each sample. Furthermore the normalization sample-to-sample using a housekeeping gene (B2M in this case) avoids the underestimation of the expression of the target gene due to dead cells, if any. Therefore it is likely that the GAPDH gene expression was referred to viable cells.

On the basis of your considerations, we have added the detailed descriptions of the procedures in "METHODS" section (pg 5 section "Total RNA expression", pg 6 " Reverse transcription" and "Real Time PCR").
ANSWER TO MINOR ESSENTIAL REVISIONS:

The phrasing and English language in the abstract has been reviewed in the "Background" and "Methods" sections as suggested.

We hope that you can find now this paper suitable for publication in BMC Cancer.

Thank you for your cooperation.

Best regards,
Luca Dalle Carbonare, M.D.

Department of Biomedical and Surgical Sciences
Medicina Interna D
University of Verona
Policlinico G.B. Rossi
Piazzale Scuro
37134 Verona - Italy
E-mail: luca.dallecarbonare@univr.it
Tel. +39-045-8074684
Fax +39-045-583041