Author's response to reviews

Title: Clone-specific Expression, Transcriptional Regulation, and Action of Interleukin-6 in Human Colon Carcinoma Cells

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Author's response to reviews: see over
Dear Dr. Lê:

We are glad that we are able to re-submit Ms. #5869405091573053 “Clone-specific Expression, Transcriptional Regulation, and Action of Interleukin-6 in Human Colon Carcinoma Cells, by W. Brozek et al. We thank the reviewers for their criticisms and suggestions, which we found very helpful in improving our manuscript.

We have asked Dr. Fekix Bronner, Professor emeritus at the University of Connecticut, USA, for help with language corrections. Prof. Bronner is a well known editor of text books and book series in the field of calcium and bone biology.

We want to note that we answered to all criticisms and made all the changes that were required by the reviewers, so that we do hope that our manuscript is now appropriate for publication in BMC Cancer.

Kind regards,

(Meinrad Peterlik)

P.S. All changes in the manuscript other than language corrections are listed on the following page.
List of changes made in ms. # Ms. #5869405091573053

Reviewer 1 (Dr. M. E. Street)

General:

We are delighted to refer to the paper by Street et al., J. Endocrinol 2003 in the revised version of our manuscript (ref. # 7).

Minor remarks:

1. Methods, 1st para and Table 1: It is unclear how alkaline phosphatase activity was measured. The incorrect reference to the paper by Stierum et al was removed. For detailed description of the APase assay in Caco-2 cells reference is made to the paper by Zucco et al. (ref. #20). Additional information is given in Methods (p. 6, para 2) and in legend to Fig. 4

2. last paragraph of results: make clear in brackets that IL-6 concentrations ranged from 0 to 100 ng/ml The paragraph was rephrased to clearly indicate that the proliferative effect of IL-6 was determined at two concentrations, i.e., 10 and 100 ng/ml (see also pt. 5)

3. Figure Legends: Fig. 5 was omitted on suggestion by reviewer 3. In addition, all speculations on a possible role of IL-6 in metastasis and secondary tumor formation were also omitted from the manuscript.

4. Figure 2A and C: Labeling of y axis was corrected.

5. Figure 3: X-axis: add IL-6 concentration (100 ng/ml) There seems to be some misunderstanding, because the labels of the histograms clearly show that the proliferative effect of IL-6 was evaluated at two concentrations, i.e. 10 and 100 ng/ml. The fact that 100 ng/ml IL-6 could be a supraphysiological concentration is mentioned in Results (p. 13, para 2).

6. Figure 4: Information on normalization of APase values was added to Figure legend (see also pt. 1)

7. „viz.“ changed to „i.e.“

8. “collated” changed to “reported”

9. “augmented” changed to “increased”

10. discussion, 1st line: “regulatability” changed to “regulation”
Reviewer 2 (Dr. H. Lahm):

Major Compulsory Revisions:

1. It is well known that the Caco-2 cell line is highly heterogenous. Also the COGA-1 cell line in our hands turned out to be heterogenous. For these reasons we had established homogenous and stable subclones from both cell lines, which we had used successfully in our previous work. Statements to indicate this fact were added to Methods (p. 5, para 1 and 2).

In addition, we want to note that the use of the colon carcinoma cell clones in the present study was deemed “useful and correct” by Reviewer 1.

2. From our previous work on the ability of colon carcinoma cells to produce IL6 (see Brozek et al.) it was clear that IL-6 expression on both the mRNA and protein level in Caco-2/AQ cells is low. Therefore, in the present study, we added a constant background of 10 pg/ml IL-6 to all samples to improve intra-assay accuracy of the IL-6 ELISA,. A statement to indicate this fact was added to Methods (p. 8, para 1).

The finding that well and moderately differentiated colon carcinoma cells produce IL-6 is consistent with previous observations in our and other laboratories. The important point however is, as it becomes clear through the present investigation, that IL-6 levels remain low in well and moderately differentiated cells even under the influence of PGE2 and IL-1β. This in our opinion is relevant for the clinical situation of colon cancer patients.

3. PCR analyses were performed exactly as described in Methods. An explanatory note how the gels shown in Fig. 1 were generated was added to Methods, as was a statement on negative controls (p.7).

4. Fig. 2B now shows CK8 transcripts for all cell lines

Reviewer 3 (Dr. C. Belluco)

Major Compulsory Revisions:

We have omitted Fig. 5 from the manuscript. “Conclusions” were rewritten according to the suggestion of the reviewer so that they deal only with the molecular mechanisms of IL-action that can explain, as Dr. Belluco states, “a more aggressive behaviour of undifferentiated tumours”.