Author's response to reviews

Title: Deoxycholate induces COX-2 expression via Erk1/2-, p38-MAPK and AP-1-dependent mechanisms in esophageal cancer cells

Authors:

Eileen Looby (loobye@tcd.ie)
Mohamed M. M. Abdel-Latif (abdellmm@tcd.ie)
Veronica Athié-Morales (amoralev@tcd.ie)
Shane Duggan (shduggan@tcd.ie)
Aideen Long (longai@tcd.ie)
Dermot Kelleher (dermot.kelleher@tcd.ie)

Version: 2 Date: 1 April 2009

Author's response to reviews:

Editor-in-Chief
Melissa Norton, MD
BMC Cancer Journal

Prof. Melissa Norton

On behalf of my co-authors, I submit the enclosed revised manuscript for consideration by the Journal as a research article. This paper has not been submitted for publication elsewhere.

Manuscript Title: Deoxycholate induces COX-2 expression via Erk1/2-, p38-MAPK and AP-1-dependent mechanisms in esophageal cancer cells.

The present study investigated the molecular mechanisms underlying DCA stimulated COX-2 signaling pathway in esophageal adenocarcinoma cells and their possible contribution to deregulated cell survival and apoptosis. Induction of the anti-apoptotic protein COX-2 by deoxycholate, via MAPK/AP-1 pathway appeared to balance the DCA mediated activation of pro-apoptotic markers such as PARP cleavage and DNA fragmentation.

I look forward to hearing from you.

Yours sincerely,

Dr. Aideen Long
Department of Clinical Medicine and Institute Molecular Medicine
Trinity Centre for Health Sciences
Trinity College Dublin
St. James's Hospital
Reviewer 1

Minor revisions:
1- A quantitative analysis has performed using densitometry for the western and gel shift data (Figure 1) to demonstrate the differences in protein expression. The quantitation of most gels is provided where appropriate, in addition the data was performed by multiple techniques to confirm the results. Also, the quality of all figures is improved.

2- A quantitative data is provided for Figure 3D.

3- With regard to Figure 4C and D actin loading and statistics, Fig. C and D are deleted because the expression of PARP was undetected at short exposure and the results at 6h-24h clearly demonstrated the change in PARP expression. The figure 4 labels and legends are also corrected.

4- The cross talk between MAPKs and different signalling pathways such as NF-kB and AP-1 are now addressed in the discussion section.

Discretionary revisions:
1- The question with regard to DCA is a major component of refluxate. The sentence is modified in the abstract line 3.

2- The comment about the potential non-specificity of the pharmacological inhibitors is addressed in the Discussion section.

3- The line of second paragraph "investigated" in Discussion page 1 and Figure 6 legend, line 7 400"box"M is corrected.

Reviewer 2

Major points:

1- A quantitative analysis has performed using densitometry for the western and gel shift data (Figure 1) to demonstrate the differences in protein expression. The quantitation of most gels is now provided where appropriate, in addition the data was performed by multiple techniques to confirm the results. Also, the quality of all figures is improved.

2. High concentrations of DCA (above 300 µM and exposure over 8h) were clearly toxic and cause cell death by apoptosis as seen in Figure 4 (A and B) compared to untreated cells. Furthermore, the bile DCA is a potent inducer of apoptosis in several cell types including, hepatocytes and colonic cells (Ref. 10, 13, 28). These results are in a parallel with the profile of apoptotic protein
expression such as PARP and caspase at the same concentration and time exposure used in our system. DCA induced DNA fragmentation and PARP cleavage, two of the hallmarks of apoptosis, in esophageal cells as assessed by ELISA and Western blotting.

3. With regard to the identification of the proteins of the induced-AP-1 DNA complex, a panel of antibodies against c-Fos, Fra-1 and c-Jun were used. We have not used a combination of anti-Fra -1 and anti-c-Jun to prove that c-Fos is not involved. However, if a combination of anti-Fra -1 and anti-c-Jun were used, this does not exclude the presence of c-Fos in the induced complex, so we had to use each antibody individually and the combination of all antibodies. A similar concept of a cocktail of antibodies against AP-1 has been used in a number of studies to prove that which component of AP-1 presents in the DNA complex.

4. We totally agree with the reviewer comment with regard to the use of pharmacological inhibitors and that molecular inhibitors could give robust result for each same pathway. All these inhibitors used in this study have been proven to be specific to each pathway such as inhibitors for ERK, p38 and caspase signalling. In addition most signalling studies use signal transduction inhibitors as a proof of concept. A sentence to indicate the concern about the specificity of these inhibitors is now made in the discussion Section.

Minor points:
1. The pages are numbered.
2. The comment made by the reviewer that studies were performed solely on a cancerous cell line and that interpretation to the in vivo situation should be taken with caution has been addressed in the discussion section.
3. PMSF and leupeptin have been used in cell lysis buffer as inhibitors of proteolysis which were enough to block proteolysis.
4. Western blot analysis method has been revised and included more details such as concentrations of primary and secondary antibodies.
5. A quantitative data for Figure 1C is now provided.
6. In most of our experiments actin and tubulin were used as loading protein controls. These proteins have been equally used as controls in many studies.
7. With regard to Figure 4 C and D for actin loading and statistics, Fig. C and D are deleted because the expression of PARP was undetected at short exposure and the results at 6h-24h clearly demonstrated the change in PARP expression. The figure 4 labels and legends are also corrected. Figure 6E clearly demonstrate the increase in PARP expression in the presence of the combination of DCA and aspirin.