Reviewer's report

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

Version: 1 Date: 18 November 2008

Reviewer: Gareth Jenkins

Reviewer's report:

The paper is well written, contains a wealth of data and covers an important current issue in neoplastic development (the role of bile acid in oesophageal cancer), as this form of cancer is increasing in incidence. The manuscript assesses the role of deoxycholate (DCA) in activating AP-1, up-regulating cox-2 and the impact this has on apoptosis. The findings are of major interest not least because cox-2 inhibition is being advocated as a chemoprevention measure and a major trial (10,000 patients) is underway in the UK. Hence further understanding of the molecular mechanisms of cox-2 expression are needed.

The overall conclusions of the paper are sound and are well supported by the data. DCA clearly activates AP-1 (fra and junB and c-jun) via a MapK pathway involving erk and p38 and concurrently upregulates cox-2 whilst also abrogating proliferation and inducing low level apoptosis.

Minor essential revisions:

My only concerns are a lack of a quantitative (and hence statistical) analysis of the western and gel shift data, there is merely a qualitative assessment in most cases. In most cases the data is clear from the gels provided, in some cases however it is not. The authors state that the experiments have been done in triplicate, I would like to see some quantitative (and perhaps statistical) confirmation of some of the less obvious effects. Some of the claimed effects are somewhat borderline from looking merely at the gels provided. E.g. Figure 1 B, the blocking antibodies to fra1 do not appear to greatly affect the Ap-1 complex individually. It may be due to the images that I have access to, so is there some quantitative data available?

In Figure 3D, there is no clear abrogation of active p38 by SB203580 at the 1 hour time point, again some quantitative data might help here. In Figure 4C, there are fewer Actin lanes (6) than PARP lanes (8) which makes it difficult to assess the loading levels (and there are some noticeable differences in actin levels). In Fig 4, 4G is not referred to in the legend, nor in the text. I think 4E is mistakenly referred to as the 0-500uM treatment, where it should be 4G. 4H is not referred to at all.

The discussion summarises the data presented and doesn't mention the potential cross talk between pathways and instead asserts a very linear model for MAPK signalling. MAPK elements are capable of activating other signalling pathways
which can exert the same effects (proliferation, apoptosis, anti-apoptosis) e.g. by cross-talking to NFkB which can exert anti-apoptotic effects. I think a more balanced discussion on this topic might be useful.

Discretionary revisions:

I don't think DCA is a major component of refluxate (abstract line 3), it is important due to its reactivity, but is still a minor component (even when including the conjugated forms). DCA is however a carcinogen (Cook et al., 1940) as well as a tumour promoter.

I think a comment about the potential non-specificity of the pharmacological inhibitors might be warranted.

Discussion page 1, first line of second paragraph "investigated"

Figure 6 legend, line 7 400"box"M

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'