Reviewer's report

Title: Homeostatic response under carcinogen withdrawal. Heme oxygenase 1 expression and cell cycle association

Version: Date: 3 September 2006

Reviewer: Inge Bauer

Reviewer's report:

General
The paper by Castronuovo describes the changes in hepatic gene expression of heme oxygenase-1, Bcl-2 and a variety of cell cycle related proteins in a mouse model of carcinogen withdrawal after occurrence of preneoplastic lesions. They report an increased expression of cyclinE/CDK2 after carcinogen withdrawal and a reduction in expression of p21, Bcl-2 and HO-1 back to normal levels. In earlier studies the authors already characterized the hepatic expression pattern of HO-1 after treatment of mice with DAB and the effect of acetylsalicylic acid on HO-1 expression and expression of cell cycle related proteins. The present paper adds information about the effect of carcinogen withdrawal on hepatic gene expression (HO-1, cell cycle related proteins). The data are highly descriptive, the authors do not provide any mechanism explaining altered gene expression.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The data are highly descriptive, the authors discuss a reduction of oxidative stress after carcinogen withdrawal leading to a reduction in HO-1 expression, but they do not provide any data on oxidative status associated with their model and the effect of carcinogen deprivation. See Page 7, third paragraph, last sentence: “probably due to a decrease in the oxidative stress”.

2. In the abstract and in the materials and methods section the authors describe the use of 18 mice. Did they use 6 mice/group or is it a different group size? In the figure legends to figures 2 and 3, they mention the analysis of 3 animals per group (“3 independent replicates”). Why did they do 6 mice/group and only analyze 3 of them and only show the result from one animal in the Western blot analysis?

3. Concerning the presentation of data on immunohistochemistry, I have some major concerns:
From the results section, the figure legends and the figure itself, it is fairly hard for the reader to identify the different conditions shown in figure 4 a-f. Please label figure 4a-f with control, HR, HC.

In the results section, the degree of HO-1 staining is given as plus and minus signs, which is a semiquantitative statement. However, the authors do not mention how they assessed “mild” or “intense” expression of HO-1 in the different cell populations. Did they count the number of HO-1 positive cells or did they measure the intensity of the staining? Please give an explanation.

Figure 4a: Figure legend to figure 4a: “and normal HO-1 positive hepatocytes”. Please explain the term “normal” in this context. Normal hepatocytes (hepatocytes in livers from healthy control animals) do not express HO-1 protein.

Figure 4 b and d: Is it the same magnification as a,c,e,f? Please indicate the magnification.

Figure 4 b: In the results section and the figure legend, the authors state that the cells marked by an arrow are liver macrophages. How did the authors identify these cells as macrophages (resident macrophages (Kupffer cells) or invaded macrophages?). Did they perform staining for surface markers of macrophages in serial sections? Kupffer cells are the only cell type expressing HO-1 protein in normal liver tissue.

Figure 4 c: In the results section, you mention HO-1 staining in hepatocytes and macrophages. Please identify hepatocytes and macrophages by arrows.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. Page 3 “Background” first sentence of the last paragraph: “cell cycle related proteins” instead of “cell related proteins”

2. Page 7, first sentence: “only partially restituted p21cip1/waf1 to basal levels”
Looking at the Western blot analysis in Fig. 2, levels in HR animals are exactly the same as in control animals, so I would suggest to change the term “partially” to “completely”.

3. Page 7 second paragraph: “BCl2 expression is highly induced”. In my opinion, an increase in expression of 33% is not highly, I therefore suggest to change the term “highly” to for instance “slightly”.

4. Figure 2: Are the samples run on the same gel? Why are the lanes cut? Why don’t you show all three samples for one condition, they can easily be run on one gel? In the densitometric analysis, why are 2 bars shown for controls, i.e. what is control-HC, what is control-HR?

5. Figure 3: same comments as for figure 2. In addition: the statistical sign *P<0.05 vs. HC group is missing in the figure.

6. In many models associated with oxidative stress, there is a cell-type specific induction of HO-1 in the liver. In addition, you find an induction of HO-1 expression in different regions in the liver. Dependent on the initiator of the oxidative stress response, you find an induction predominantly in the periportal or pericentral region of the liver. From your immunohistochemical analysis it is not clear, whether such an expression pattern exists in your model. Do you have evidence for the existence of a regional expression pattern of HO-1 after treatment with DAB?

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests