Reviewer's report

Title: Enhancing chemosensitivity to gemcitabine via RNA interference targeting the catalytic subunits of protein kinase CK2 in human pancreatic cancer cells

Version: 1 Date: 18 April 2010

Reviewer: Godefridus Peters

Reviewer's report:

The authors investigated whether the efficacy of gemcitabine could be enhanced by modulation of various subunits of CK2. CK2 plays a role in cell division and proliferation. Although there was earlier report on gemcitabine and CK2 it was not particularly clear to me why the authors chose to investigate this combination specifically in pancreatic cancer. A real mechanistic rationale was not given, except on p. 5 where a general description was given for each drug. The authors postulate that especially downregulation of the #“ subunit may enhance the efficacy of gemcitabine. The paper needs revisions in view of presentation of the data and explanation of the results. The data are not explaining an interaction between gemcitabine and CK2# inhibition; it is not clear to me whether there is an interaction (in case of cytotoxicity, the effects should be more then additive) or whether the effects are additive, which probably means no interaction.

Results: Major Compulsory Revisions

p. 9-10: an extensive description (almost each concentration of each cell line) of the sensitivity of cells to gemcitabine is given. This is redundant; Fig 1 and Fig 2 should be exchanged; first give growth inhibition and subsequently the associated disturbance in cell cycle.

Fig. 2: it is unclear to me why no normal growth inhibition curves are found in Fig. 2a, although a concentration effect was seen in Fig 2B. E.g. PANC-1 shows no growth inhibition (Fig 2A), but shows inhibition of BrdU labelling and disturbance of cell cycle. Possibly the WST-1 is not the most suitable assay for this purpose or the cells did not grow sufficiently during the assay. What is the doubling time? I prefer to get a list of IC50 values.

Fig. 1A: it is strange that there are no cells in the S-phase of MaPaCa cells. What is the purpose of Fig 2B; it only shows that there are less cells when treated with gemcitabine.

In Fig 3C cell death of 7% is reported but this is not in agreement with the number in Fig 1A, which is just 2-3%.

p. 11: do not give number of lanes in the text; the effects are difficult to follow. Clearly indicate treatment in the figure. I do not agree that cells are efficiently killed in the combination, actually cell kill in the combination is less then what can theoretically be expected from the effect of gemcitabine alone or anti-CK alone.
p. 13: the effect on JNK and of the JNK inhibitor SP600125 is interesting, however, it does not provide a sufficient explanation; the authors claim that gemcitabine-induced cytotoxicity is mediated by CK2# mediated JNK signalling. However, the authors did not investigate cell death in the presence of SP60012, only an effect on signalling was investigated.

p. 14: specify what was done in vivo, clarify in vivo as well?

Discussion: Major Compulsory Revisions

This part is too speculative, based on results not presented:

p. 15: cell death is postulated to be increased, but as indicated above, I believe it is only additive or even less then additive. The JNK pathway may be of interest, but essential experiments have not been done.

Last paragraph: the authors mention that “the gemcitabine resistance mechanism” is with Akt; although there is an interaction, this is only one out of many possibilities. Pancreatic cells may have a decreased uptake of gemcitabine, a decreased phosphorylation or better repair of DNA damage, which may be more important.

Figures (Major Compulsory Revisions):

Figure 1: why are the bars not the same; they do not all reach 100%, especially in the BxPC3

Figures 2, 4 and 6 are unreadable. Remove the horizontal grid, because this makes the letters unreadable; next use a better readable font and increase size of the legend.

Fig. 2: growth inhibition should not be given in bars but as curves similar to presentation of the NCI of growth inhibition; the figure can then be simplified, with 4 lines in one figure.

Figures 3, 4, 5 and 6: the explanation of the numbers is given in one figure, but when one looks at the subsequent figures, one has to refer to the previous legends or even the text in the paper. Each figure should have an adequate description of each lane in the western blots, not based on numbers; each figure should be self-explaining without reference to another figure or text.

Check citation of ref 14: Y de CW, this seems to be incorrect.

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

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'