Reviewer's report

Title: TUCAN/CARDINAL/CARD8 and apoptosis resistance in non-small cell lung cancer cells

Version: 1 Date: 21 February 2006

Reviewer: Rajagopal Ramesh

Reviewer's report:

General
In the submitted manuscript Checinska et al., have investigated the role of TUCAN in drug resistance in non small cell lung cancer cells (NSCLC). In this study the authors have examined the expression of TUCAN and its downregulation in NSCLC to sensitize to Cisplatin. Although the results are convincing several deficiencies have been identified that will need to be answered prior to acceptance of this manuscript.

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

1. Figure 1 - The authors in the results section (pg # 9) and discussion (pg # 13) state that caspase-9 activation was not seen in H460 and GLC4 cells after CDDP treatment but could see only after addition of cytochrome C and dATP. Although this statement can be assumed to be somewhat true, it is not clear whether the activation observed in cells treated with CDDP alone was significant compared to untreated cells. Per Figure 1C, CDDP treatment alone induced 1.5 times cleavage compared to untreated controls (0.5 times increase). If this is statistically significant then the wording has to be rephrased. Additionally, in Figure 1B semiquantitative analysis of the change in caspase-9 levels would be of help in examining the results of Figure 1C. For example, if the change/increase in caspase-9 levels in Figure 1B at 48 h (time point common to both Figure 1B and 1C) is 1-2 fold in cells treated with Cyc.C/ATP compared to those that were not, then the Caspase-9 activity in Figure 1C would correlate as there is an approximate 2 fold increase in activity. The authors should do this comparison and rephrase the sentence in the results and discussion section. This may also change the interpretation for this figure though not the final conclusion.

2. Figure 4 - the authors in the results section for figure 4A (pg. 12) indicate that mRNA for TUCAN in cells stably expressing TUCAN SiRNA was inhibited by 70%. However, per Figure 4A it appears that inhibition of mRNA is not greater than 60%. This needs to reexamined and corrected. Additionally, it is recommended that the authors include a figure showing downregulation of TUCAN protein expression in addition to mRNA inhibition.

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. At what time point the CDDP was added to the cells and how long the cells were treated prior to analyses for SubG1 population (Fig. 4B)?

2. It would be better if the authors provide a rationale in the methods or results section for using IC80 rather than IC20 or IC50 in the study.

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Discretionary Revisions (which the author can choose to ignore)

1. Have the authors tested treating the cells stably expressing TUCAN SiRNA at IC20 or IC50 rather than IC80 as the differences in restoration of CDDP sensitivity following downregulation of TUCAN might have been missed at IC80.

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

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interest.