Reviewer’s report

**Title:** Enhancer of zeste homolog 2 (EZH2) expression is an independent prognostic factor in renal cell carcinoma

**Version:** 1  **Date:** 17 May 2010

**Reviewer:** Kiyohide Fujimoto

**Reviewer’s report:**

**Summary**
In the clinical management of high-risk renal cell carcinoma (RCC), accurate evaluation of prognosis is an important issue. In this paper, the authors investigated whether the nuclear expression of enhancer of zeste homolog 2 (EZH2) protein is a clinically useful biomarker for RCC patients who underwent nephrectomy. Polycomb proteins play important roles in the epigenetic gene regulations and are considered to contribute to cell proliferation and tumorigenesis. The present study has been carefully conducted based on the remark criteria to allow adequate and reliable assessment of the results obtained from this study. As a result, the authors revealed that EZH2 was a powerful independent prognostic marker of unfavourable cancer-specific survival in patients with metastatic and non-metastatic RCC. These findings might enable physicians to select the optimal therapeutic strategies.

**Comments**
1) The authors describe that only 10 patients had high (>50%) nuclear EZH2 staining. The number of patient in this group seems to be small to adequately perform the multivariate prognostic analysis. In fact, there were no statistically significance factors in analyzing this group. What was the reason for setting cutoff percent of EZH2 expression at 50% and 25%?

2) Is there a significant difference between expression levels of EZH2 in the periphery and in the center of tumor? Previous reports described that EZH2 was related to invasion and progression of cancer cells. These results supported that EZH2 would be expressed predominantly in cancer cells located in expanding peripheral zone. The authors need to describe the text about this issue.

3) Karnofsky PS, delay between diagnosis and treatment, serum LDH, corrected calcium and hemoglobin are widely known to be powerful prognostic factors in metastatic RCC. These variables should be included into the analyses for evaluating the true prognostic value of EZH2 expression in metastatic RCC (Table 4). The authors have to describe about this issue.

4) There is no explanation for Figure 1A in the text. This should be mentioned in the text.

5) In the section of Conclusion, the authors described that inhibition of EZH2
expression might be a novel therapeutic target. However, EZH2 was expressed in infiltrating lymphocytes, proximal and distal tubule epithelial cells, proliferating parabasal cells in the normal cervical epithelium, proliferating cells of normal mammary gland tissue and mammary stem cells. Inhibition of EZH2 seems to potentially lead important human organs to various and undesirable toxicity if the authors have and use small molecules or neutralization antibody for targetting EZH2

Minor Criticisms

1. Page 8 Line16, the abbreviated word “CCS” should be ‘CSS’.

2. Table 2. A total number of patients in the sex row, the columns of >25-50% and >50% are incorrect.

3. Table 2. How about EZH2 expression of papillary and chromophobe RCC? These data are important to discuss tumor aggressiveness in the consecutive histological types.

4. Figure 1. The magnification should be mentioned.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

'I declare that I have no competing interests'