Reviewer's report

Title: Prevalence of Von Hippel-Lindau gene mutations in sporadic Renal Cell Carcinoma: results from the Netherlands Cohort Study

Version: 2 Date: 8 February 2005

Reviewer: Peter Schraml

Reviewer's report:

General

In this report van Houwelingen et al. analyzed paraffin-embedded tumors from 235 renal cell cancer patients for VHL gene mutations using SSCP followed by direct sequencing. They found VHL mutations in 61% clear cell RCCs, which is higher compared to previous studies. VHL mutations were not only restricted to clear cell RCC but were also seen in oncocytoma, as well as in chromophobe, papillary, and unclassified RCC. In some cases different paraffin blocks obtained from one tumor showed different mutations while in other tumors several mutations were seen in one sample. The authors found no significant correlation between tumor parameters (grade, stage, nodal status, size) and mutated/wildtype tumors.

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

1. It is not clear how the authors verified VHL missense mutations. For example, two separate PCRs would clearly demonstrate whether or not the mutation is true (same mutation in two PCR products or artefact in one sample and no or another mutation in the second one). Therefore, forward and/or reverse sequencing of a shifted PCR product, as described by the authors, is not sufficient especially if formalin-fixed paraffin-embedded material from 52 different labs(!) is used. This would also explain the uncommonly high rate of VHL mutations in clear cell RCC and the observation of different mutations in different paraffin blocks from one tumor.

2. The authors classified tumor stage according to the 1987 revision of the UICC-TNM classification. The last (5th) edition of the staging system of malignant tumors appeared in 1997 and is commonly used nowadays. Compared to the former edition there was a dramatic change in the pT classification of organ-confined renal cancer in which the break point between category pT1 and pT2 was increased from 2.5 cm to 7 cm. The introduction of the new cut point brought considerable changes to the number of cases in each category, with more cases now being categorized as pT1. It is also important to be aware that the new pT stage is strongly correlated with patient survival. The use of the new system might therefore have great influence on the calculations regarding correlations between tumor stage, patient outcome and VHL mutation.

3. Obviously the authors have access to additional information including follow-up data, lifestyle, and dietary habits of cancer patients. A possible correlation between VHL mutations, survival and risk factors would strengthen the impact of this paper.

4. Samples having at least 10% tumor cells were considered for sequence analysis, which is very uncommon. Does this mean the authors were still able to detect VHL mutations in heterozygous tumors with only one allele being mutated? How many tumors consisted of only 10% malignant cells? How did the authors retrieve the material from these samples (microdissection, scraping)?
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. Grammar and spelling should be re-checked (e.g. subtypes will be assessed... on page 11).
2. SSCP analysis is a well described and accepted tool for mutational screening. SSCP analysis and findings should be written as concise as possible. Table 2 and 3 should be omitted.
3. The distribution and types of VHL mutations from all tumor subtypes should be listed in one table. Table 4 and 5 should be combined (without data about sex, age, and % tumor tissue). Tumor parameters (stage, grade, nodal status) should be briefly summarized in an additional table. Table 6 is too complex and should be omitted.
4. There is no significant correlation between tumor size and VHL mutation. Therefore, the result should be briefly mentioned in the results section.

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.