Reviewer's report

Title: Differential expression of a new isoform of DLG2 in renal oncocytoma

Version: 1 Date: 17 February 2006

Reviewer: Andrew Young

Reviewer's report:

General

The authors identified a sequence interpreted as a new isoform of DLG2, containing 3' DLG2 exons and the hypothetical gene sequence FLJ37266. The authors state that coding regions for DLG2 and FLJ37266 are located nearby on chromosome 11q14.1. The DLG2/FLJ37266 sequence was differentially expressed in study specimens of renal oncocytoma (RO) compared to normal kidney and several malignant renal tumors. In particular, it was significantly overexpressed in RO versus chromophobe renal cell carcinoma (CH-RCC). Differential expression was discovered with whole-genome oligonucleotide microarrays, and confirmed with RT-PCR and Northern blot. The sequence was further characterized by 5' and 3' rapid amplification of cDNA ends (RACE).

The authors are established experts in molecular classification of renal tumors, including RO and CH-RCC, and make use of strong local research infrastructure including clinical tissue banking facilities. They justify the significance of this work in terms of the difficult differential diagnosis of RO versus CH-RCC and other renal tumors.

Identification of specific markers for RO is an important clinical priority, making this article of potential great significance. The basic significance of DLG2 and/or FLJ37266 in kidney or cancer pathobiology is not yet known, and cannot be assessed from this work. Since the immediate impact of this paper would likely be in clinical translational research, certain issues need to be addressed to confirm the significance of the findings (see below).

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

1. For the initial microarray experiments, the authors appear to have pooled 3-5 cases from each diagnostic class (normal kidney, oncocytoma, and several subtypes of renal malignancy). As far as can be told from the methods, the same RNA pools were used for confirmation by RT-PCR and Northern blot. Therefore, it cannot be known if differential expression of DLG2/FLJ37266 is due to a single outlier case or is a consistent finding in renal tumor specimens. Clinical significance cannot be assessed until the finding is validated in a larger independent study cohort. Additional frozen tissues could be assessed with the assays developed in this study. Fixed archival tissue would be more representative of clinical diagnostic settings, and could be amenable to quantitative RT-PCR or immunohistochemistry if an antibody is available.

2. The authors state that DLG2 and FLJ37266 are closely linked on 11q14.1, but do not include a reference. The original mapping of DLG2 was to 11q21 [Stathakis DG, Lee D, Bryant PJ. Fine-scale physical map of the 11q21 region surrounding the human DLG2 locus, the gene encoding Chapsyn-110. Genomics 54:186-8, 1998]. The current UniGene entry for DLG2 also locates the gene to 11q21. Has the accepted location of DLG2 changed? If so, please provide reference. If not, could linkage of DLG2 and FLJ37266 represent a genomic alteration? As discovered in large part by the authors, 11q is a recurring but not universal mutation site in oncocytoma, further underscoring
the concern that expression data is due to a unique outlier specimen.

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. Figure legends could be more explicit. For Figure 3 legend, the term "...for the exons 22-13..." may need to be changed to "...for the exons 22-15...". For Figure 4 legend, please state which tumor(s) and normal specimen(s) the labels "T" and "N" represent.

2. On page 4, paragraph 3, the phrase "...they were not detected..." should be changed to "...they were not detected..."

Discretionary Revisions (which the author can choose to ignore)

1. The authors use of the Heidelberg renal tumor classification system is quite understandable. The WHO classification system is more recent. The difference is very unlikely to result in changes in classification of the study specimens. Still, the authors may want to reference the WHO as well.

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: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.