Reviewer's report

Title: Identification of a region required for TSC1 stability by functional analysis of TSC1 missense mutations found in individuals with tuberous sclerosis complex

Version: 1 Date: 22 June 2009

Reviewer: Aristotelis Astrinidis

Reviewer's report:

In this work, Mozaffari and colleagues perform functional assessments of TSC1 (hamartin) missense mutations. Although numerous TSC2 missense mutations have been described to date, TSC1 missense mutations represent a small fraction of disease-causing mutations for Tuberous Sclerosis Complex. The authors show that non-functional TSC1 missense mutations are clustered in the N-terminus region of the protein (first 270 amino-acids); missense mutations in other regions of the protein were fully functional. Based on sequence analyses of TSC1 between species, the authors conclude that the middle part of TSC1 (amino acids 270-680) is substitution-tolerant - this finding is important for future studies of TSC1 function. Very strong conservation was observed in the last approximately 80 amino acids of TSC1.

Overexpression of certain mutants (L50P, L61P, L93R, V133F, R190P, and the previously described L117P) resulted in increased pT389-S6K1, compared to wild-type TSC1, which was correlated with decreased TSC1 protein level of these mutants. For two of these mutants (L50P and L117P), the decreases protein levels were associated with degradation by the proteasome (rescued by MG132). Was this observed for the other mutants that exhibit low protein levels (L61P, L93P, V133F, R190P)?

Immunocytochemistry showed that these mutants have diffuse cytoplasmic localization when overexpressed alone, while the wild-type TSC1 has punctate/aggregate localization (as observed previously). Since localization/aggregate formation can be affected by level of expression, could this apparent difference in cytoplasmic localization of the mutants be attributed to the low protein levels observed for these mutants? E.g. could MG132 rescue the localization of the mutants, when they are overexpressed alone? This could help determine whether the TSC1 missense mutants under investigation are dominant-negative - this is true, at least in part, for the ability of the mutants to regulate mTOR signaling, since in the presence of MG132 the mutants failed to inhibit pT389-S6K1.

Discretionary Revisions

1. Data in Figure 3 (panels B and C) could be presented in an order to correspond to the way groups are presented in the immunoblot (panel A): "+insulin/MG132", "+MG132", "+insulin", "basal". This could help the readers.
2. Table 1. Same with order of mutants R190C and R190P. R190P is first, and R190C is second in Figure 1.

3. Figure 1C. A key to SIFT analyses results (although described in detail in figure legend) could help.

4. Figure 2B and 2C. For those samples that are statistically different from wild-type TSC1, asterisks should be included in graph.

5. Figure 3B-3C. Statistical significance (if present) should be indicated.

5. Figure 4. Scale bar on micrographs is missing.

**Level of interest:** An article of importance in its field

**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