Reviewer's report

Title: Conditional Disruption of Interactions Between G(alpha)i2 and Regulator of G Protein Signaling (RGS) Proteins Protects the Heart From Ischemic Injury

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Reviewer: Ulrike Mende

Reviewer's report:

This study is an extension of the authors' previous work on the consequences of disrupting the interactions between RGS proteins and Gai2 protein, using a RGS-insensitive mutant of Gai2 (G184S). They previously showed protection against ischemic injury in a mouse model with non-conditional Gai2G184S knock-in, along with other cardiac and non-cardiac phenotypes. In this study, a new Cre-ERT2-based Gai2G184S knock-in mouse model was generated with tamoxifen-dependent conversion of endogenous to mutant Gai2 in several tissues, including the heart. Tamoxifen-treated Gai2 G184S knock-in mice showed enhanced LPA-induced inhibition of cAMP production in adult cardiac fibroblasts, reduced infarct size and improved developed pressure after ischemia/reperfusion.

The authors conclude that acute expression of Gai2G184S in adult mice is sufficient to induce cardioprotective effects, which has greater potential therapeutic significance than the previously reported similar effect in mice with non-conditional embryonic and chronic Gai2G184S expression.

The new model is an advance over the existing model because it allows for temporal control of RGS-insensitive Gai2 expression (via tamoxifen administration as shown in the present study) as well as spatial control (by restricting Cre expression to tissue/organs of interest). The authors show that the expression of mutant Gai2 in the conditional knock-in model is much lower compared to endogenous Gai2, and acknowledge that this could be due to the removal of several introns and the presence of LoxP and Flp sites in the targeting construct. Despite this caveat, this model should be a useful tool for advancing understanding of the role of Gai2 and its interactions with endogenous RGS proteins in the heart and other organs.

Minor Essential Revisions:

1. A “deleter mouse line” with generalized tamoxifen-dependent Cre expression was used to assess the effectiveness of endogenous to mutant Gai2 conversion in various tissues. What was the rationale for using a generalized knock-in model for assessing the cardioprotective effect? Utilization of mice with cardiac-restricted Cre-ERT2 expression could alleviate questions about if and to what extent Gai2 G184S knock-in in non-cardiac tissues may contribute to the observed phenotype. Please discuss.

2. In addition to the developed pressure, other important functional
measurements obtained from the Langendorff preparations (including +dP/dT, -dP/dT, EDP and heart rate) should be provided and discussed for a more complete picture of the cardioprotective effect.

3. The 1st sentence in the abstract ("Regulator of G protein signaling (RGS) proteins suppress G protein coupled receptor signaling by catalyzing the hydrolysis of Gai-bound guanine nucleotide triphosphate.") could be wrongly interpreted as if all RGS proteins suppress Gi signaling and/or as if RGS proteins only suppress Gi signaling. Please rephrase to clarify.

4. Please clarify the last sentence on page 12: "Blots were hybridized to 5’ and 3’ probes, EcoNI (13.0 kb fragment) (Fig. 1D) and AflII -digested genomic DNA (Fig. 1E, this probe hybridizes to a 7.9 kb fragment from the wild type Gai2 allele and an 11.5 kb fragment from a correctly targeted Gai2 allele), respectively."

5. Please relate the results from the cAMP assay in adult fibroblasts from the conditional knock-in model to published data from the non-conditional model.

6. The genotypes of mice used for Western blot analysis shown in Figure 4 are inconsistent and must be reconciled between the result section (Page 17) and Figure 4A-C (are the bar graph annotations truncated?)

7. The labeling above the molecular weight markers in Figure 4D should be corrected to “Mr (kDa)”.

8. Please fix the following additional minor errors: (i) The comma on lane 5 of page 17 should be a full stop; (ii) “Western” in the legend for Figure 4 should be capitalized; and (iii) “derivation” in the legend for Fig. 5 should be “determination”.

Discretionary Revisions:

1. The marked change in infarct size could be visually supported by representative images of infarcted hearts from each group.

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.