Reviewer's report

**Title:** Glucocorticoids with similar receptor potency generate unique gene expression and biological pathways in human trabecular meshwork cells.

**Version:** 1  **Date:** 4 April 2009

**Reviewer:** Thomas Yorio

**Reviewer's report:**

This paper attempts to demonstrate that different glucocorticoids produce common as well as a set of unique gene arrays in trabecular meshwork cells (TM) that may be related to the various glucocorticoid receptor isoforms that are expressed in trabecular meshwork cells. There are a number of concerns with this manuscript as noted below.

**MAJOR Revisions**

1) The dose of glucocorticoids used in this study 1 uM is 1000x the affinity constant for these compounds. At such a high dose there may be actions normally not activated at typical physiological concentrations of cortisol or even what has been shown for functional responses to exogenous glucocorticoids (1 nM).

2) The authors only selected one time of incubation of 24 hr, yet many of the functional responses for glucocorticoids require several days of treatment (48 to 72 hours). The authors should do a time course for their responses. There may be differences in gene expression with the different glucocorticoids over time.

3) The authors only used two cell lines from two individuals of different ages. Therefore the gene profile is an n=1 for each age. Not sure comparative data has meaning for n=1. Additional cell lines at the similar age brackets would provide additional support for the authors claims from the gene profiling.

4) The Western blot of isoforms is not very convincing, there are numerous bands in the gel and the authors are claiming different isoform expression for some of the bands not for others. What controls were run to determine if this is indeed the proteins they think they are? What was the loading protein amount and how was this standardized and normalized? The authors use terms like higher levels, Westerns are not quantitated. What was used to correct for differences in loading. How certain are the authors that the antibodies will recognize all these isoforms? Are there selective antibodies, or control proteins.

5) The Hela cell data was following only 5 hr treatment. Why the difference in treatment time to TM cells?

6) The pathway figures at the end are somewhat arbitrary judging from the numbers of genes that are increased or decreased in the arrays. Not sure what added value they have to the manuscript at this stage of identification. There was no confirmation of the results using other RNA technology.
MINOR Revisions
Check spelling throughout manuscript

**Level of interest:** An article whose findings are important to those with closely related research interests

**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.