Reviewer’s report

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

Version: 1 Date: 8 April 2009

Reviewer: Ted S Acott

Reviewer’s report:

This is a very interesting study that could make significant contributions to this field, if several very significant concerns were adequately addressed.

Major compulsory revisions:

Overall concerns - the authors need to carefully re-read their manuscript and re-review their data to develop an hypothesis for this study. At this time, observation that the different GCs have different expression profiles in different cell lines of different ages with similar binding affinities and similar promotor activity remains a novel observation with no conceptual or mechanistic basis and numerous internal inconsistencies. Hence, the study has yet to be analyzed by the authors and processed into a manuscript. This is too bad, because it appears that this is a potentially very interesting study.

1) presumably, someone has done some studies somewhere in some tissues with these three GCs and presumably, someone has done some studies in some tissues suggesting that the various GR isoforms have some reason for existence beyond redundancy. If either of these is true, this information should be incorporated into the manuscript and used to set-up this study. If there is no literature out there on either of these topics, this should also be incorporated into the manuscript to give it some context.

2) showing similar binding affinities for Dex, FA and TA in some lysate from some cell without even mentioning the GR isoforms used and present requires that you either think that the different GCs all bind similarly to all of the GF isoforms. Presumably, the binding affinity you measured is a conglomerate affinity created by the different GR isoforms in the lysate you used, if the different isoforms might have different binding affinities. What this measurement has to do with the binding affinities in TM cells is also not clear nor discussed candidly.

3) showing similar promoter actifity for one specific GC element in one cell type is similarly uninformative relative to TM cells. You show that they have different GR isoform ratios. Both 2) and 3) suggest that you should not have obtained the results from the microarray studies that you observed. Probably, if you rewrote the setup, point and conclusions from these two experiments, they would help in defining/interpreting what the microarray data really mean. - there is probably an hypothesis in there somewhere and this is an interesting although obscure set of observations.

4) You need to carefully look at your microarray data and draw information from
them and develop some conclusion beyond what the analysis programs tell you the point is. The Ingenuity and GoMiner programs are clever, but not really very smart. You need to also look at the data and see what it means.

5) CG effects on many gene expression patterns, both from the pre-array days and since, have been studied extensively. Do the three CGs change expression of genes that are well known to be affected in TM and other cells by CG treatment? Are the genes in the little light purple central overlap (1968 & 1150 genes) typical CG changed genes? Granting that the different CGs and different combinations of GR isoforms may have interesting different effects on expression profiles, they should have lots of very consistent, common and well-studied overlaps. This aspect of the data analysis is absolutely critical to establish the degree of credibility of the study and to put it into perspective.

6) CG effects, particularly Dex, have been studied extensively in TM cells. You need to carefully and with specific genes in mind, relate your observations/data to the literature. This was done only in broad strokes and in a fairly cursory manner and is very important to placing this study in context.

7) either via a Venn as in fig 4, or at least discussion wise, the degree to which the two TM lines exhibit overlapping or non-overlapping expression with the various CGs needs to be discussed. The pathway differences suggest that in these two cell lines, everything is different. Hopefully, this is not really the case and the pathway differences you list were discussed because they were interesting differences. Are there many or few genes which overlap between the two cell lines when treated with Dex, or with TA or with FA? If there is not considerable overlap, I worry about the reality of your studies. Do all the Venns and talk about/summarize them, although you probabl don't need to show all the various possibilities.

8) it is notable that many of the highlighted genes in the various pathways are the same genes, like Jun.

9) the duscussion really needs major work - do you think the different CGs bind differently to different GR isoforms? could they just have different effects on the same isoforms? there are things you can conclude from these studies and things you can just suggest as possibilities - using what is known in the literature (which this reviewer does not know) and the possible ways these expression differences could be occuring, this paper is potentially much more interesting than the authors present/show it to be.

**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