Reviewer's report

Title: MicroRNA-125a is overexpressed in insulin target tissues in a spontaneous rat model of Type 2 Diabetes

Version: 1 Date: 9 November 2008

Reviewer: David Arthur Carlyle Simpson

Reviewer's report:

This is a thorough study of the changes in miRNA and mRNA expression in the chosen model system and a good attempt to relate these findings. However, the following points should be addressed.

• Major Compulsory Revisions

1. The authors compare the GK rat with the BN rat, which is described as ‘genetically distant’. The GK rat is derived from the Wistar rat, which has been used as a control strain in previous gene expression studies, eg by He at al. The authors should explain why they used the BN rat as a control and address the issue that the observed differences in miRNA and mRNA expression may be due in part/largely to strain differences rather than diabetic effects. Many studies have reported significant strain-specific differences in mRNA expression (eg Kappeler L, et al J Neuroendocrinol. 2006 Jun;18(6):426-33. Johannesen J, et Autoimmunity. 2003 May;36(3):167-75.).

2. If it is the case that strain differences may be contributing significantly to any changes observed due to hyperglycaemia, perhaps the principal finding of miR-125a over-expression in diabetic conditions could be strengthened by confirmation in another model system.

3. The miRNA microarray data have been thoroughly analysed, however is it appropriate to classify a miRNA as altered if one of 4 probes shows a change in the opposite direction (p5 line 21)?

4. In the introduction the authors quote the work of Vasudevan et al (ref 2) showing that miRNAs can up regulate translation. Whilst this is valuable work it is one of very few studies showing that miRNAs can upregulate mRNA expression, whilst there is a huge body of work confirming that miRNAs can reduce target mRNA expression. In the results section ‘differentially expressed ‘ genes are analysed together, rather than those altered in one direction. The authors should explicitly analyse the genes altered in the opposite direction to miR125a expression as has been performed in many previous studies. If a significant over-representation of miR125a targets is only observed when all genes altered in either direction are considered together it should be made clear that this is major difference from most previously published work and the implications discussed. The fact that it is all differentially expressed genes, rather than those lower in the presence of higher miR-125a, which show an over-representation of
miR-125a target sites should be made clear in the abstract.

• Minor Essential Revisions

• Discretionary Revisions

1. P6 line 24
   • ‘but no functionally effective miRNA:mRNA interaction…’
   • Suggest change to: ‘but no functional miRNA:mRNA interaction…’

2. Introduction line 7.
   • Ref 5 is excellent for the discovery of animal microRNAs, but others are more appropriate for the demonstration of miRNA action upon mRNA levels (eg 6)

3 Discussion:
   • ‘This is exactly comparable with the only previous study of miRNAs in T2D, which identified a 1.5
   fold upregulation of miR-29a, together with the paralogs 29b and 29c, in microarray analysis of skeletal muscle from GK and Wistar rats (normoglycaemic controls)’
   Add in citation to ref 17 at end of sentence

4. ‘...and the up-regulation of miR-29a in liver from the GK rat [44] are confirmed.
   Should this be ref 17 not 44?

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