Reviewer's report

Title: CRISPR-Cas9-mediated knockout of SPRY2 in human hepatocytes leads to increased glucose uptake and lipid droplet accumulation

Version: 0 Date: 27 Aug 2019

Reviewer: Flavio Cadegiani

Reviewer's report:

Overall comments:

I congratulate the authors for this great work. Authors were able to:

1. Bring extremely relevant data for the understanding of the molecular mechanisms of BF distribution and metabolic functions of the hepatocytes

2. Perform a molecular/genetic (basic) study with appropriate assumptions and background

3. Write a text easily readable, with a step-by-step logical sequence to justify their experiments

4. Employ precise assessment methods, and evaluate the most important correlated pathways

5. Discuss their findings in such correct and logical manner, without an overestimation of their results

6. Provide conclusions exactly according to their findings and discussions and provide practical applications

7. Simplify very specific molecular and genetic concepts in order to provide readers an easy understanding of such difficult manuscript for those unfamiliar with basic endocrinology, and at the same time maintain an excellent level of precision

8. Finally, be a great example of the movement towards translational medicine, linking basic and clinic endocrinology in an outstanding way

I the only reasons that I classified this manuscript as needing a major review are due to the methods section and a specific aspect of the discussion.

Methods section -
Although the methods section does not contain any error, and are correctly and more than sufficiently described, from a more comprehensive perspective I give some suggestions regarding its structure, as below:

The specific methods for each step of the experiments are adequately described.

However, I miss an overall explanation of why these specific experiments were performed, and a logical sequence between these steps. In this sections, authors have only described each experiment separately, without a logical justification for each. Many of these justifications are actually located in the results. These would better fit in the methods.

An example:

Lines 272-275 - "we hypothesised that key cellular signalling pathways may be affected in these cells. To investigate this, we carried out a phospho-kinase array for the simultaneous determination of phosphorylation levels in 43 protein kinases across multiple pathways relevant to metabolism and insulin resistance (including Akt, MAPK, mTOR and Jak/STAT signalling)."

Although this is in the results section, it should've been moved to the methods, while each experiment should've be included into a logical context. I suggest that other sentences are moved accordingly.

A logical sequence similar to how the introduction and background was performed will make the manuscript more friendly to read and bring together those who consider basic endocrinology something far from their abilities. This is important for the movement for a translational medicine. A redesign towards an easier understanding, at the same time maintain all the detailed description of each procedure, would be highly appreciated.

Discussion section -

Given the increased lipid droplet accumulation, I missed discussion regarding the potential roles in the pathogenesis of the non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). I recommend hypothesizing the possible indirect contributions of the SPRY-2 for these diseases, since it has been clearly shown its direct effects on lipid accumulation in hepatocytes and cholesterol biosynthesis, and both NAFLD and NASH are emerging conditions that may be currently contributing to an epidemic of hepatic failure.

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There are some minor comments, as found below:

Line 73 - T2DM (more commonly used than T2D) is in indirect leading cause of death due to obesity. The most T2DM-related cause of death is also CVD. It means that in the end CVD is the
leading cause of death direct- and indirectly related to obesity. I suggest a slight change in this sentence.

Another suggestion (to be optionally adopted by authors): the leading causes of chronic kidney disease (CKD) are T2DM and hypertension, and has become an epidemic, leading to an exponential growth of the need for kidney replacement therapies. The major cause of these two diseases is obesity. It is estimated that obesity may be an indirect but the main underlying cause for the epidemic of kidney failure and need for dialysis. This could be included, since despite its huge importance, few papers on obesity mention this.

Lines 93-95 - Authors properly explain the major processes mediated by the RTK signaling pathways. However, it may be interesting to include other mediations, including cell-to-cell communication, metabolism, and survival, since the tyrosine kinase receptor is amongst the most important type of receptors. The function evaluated by the present manuscript shows a metabolic, not a proliferation or differentiation role, of the RTK. I also suggest authors to exemplify relevant types of receptors that are RTK. It is important to detail the level of importance of this class of receptors for those readers that may not automatically link the RTK with some of their crucial roles.

My suggestion is to slightly change the sentence to:

"Sprouty proteins are negative regulators of receptor tyrosine kinase (RTK) signalling pathways [10], which mediate key cellular processes in a wide range of activities, including critical roles in proliferation, communication, and differentiation, but also has influences in motility, metabolism, and survival [11].

I would also include in the sentence the following suggestion of exemplification: "Several families of growth factor receptors, including vascular endothelial growth factors (VEGFs), insulin-like growth factors (IGFs), fibroblast growth-factors (FGFs) and platelet-derived growth factors (PDGFs), and that are key regulators of a variety of cellular processes, are examples of types of RTK"

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
Yes

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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