Review of: "Estrogen-related receptor alpha and Rplp1 ribosome protein-dependent translation coordinately regulate starvation response and decrease NASH progression"

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The paper “Estrogen-related receptor alpha and Rplp1-dependent translation coordinately regulate starvation response and decrease NASH progression” by Tripathi et al represents a magnificent body of work to describe the transcription factor, Esrra, in fasting/starvation and during non-alcoholic steatohepatitis. From the literature, Esrra transcriptional factors are involved in many cellular activities, thus the authors have a high bar to prove their hypothesis that the downregulation of Esrra is involved with the phenotype of NASH. To investigate these effects, the authors compare human primary hepatocytes and a mouse hepatocyte cell line under serum starvation followed by proteomic analysis. Esrra was identified and subsequent proteins containing Esrra promoter regions (Rplp1, Lamp2, Ctsd) were also evaluated under serum starvation to see if levels increased or decreased. KEGG analysis reveals that Esrra is involved with autophagy and protein translation pathways and they settle upon a set of markers demonstrated in Fig. 3 to measure these pathways in various assays in which they use Esrra knockouts or overexpression, pharmacological inhibition using C29 with the use of AML cells or in mouse livers. To investigate NASH, they used human liver biopsy samples and measured levels of their established protein markers (ESSRA, RPLP1, etc) and followed up with mouse models of NASH using Western diet and methionine-choline deficiency (MCD) diet which promotes chronic inflammation. The comparison of human, mouse Western diet, and mouse MCD diet was beautifully done in supplementary Fig. 4 and I thought that should have been in the main figures. Digging further into the effects of fast-feed cycles during NASH with the use of Esrra inhibitor, C29, to assess autophagy and protein translation made the paper very translatable to human disease. With the use of human samples, various animal models, pharmacological and genetic inhibition and activation to prove their hypothesis that Esrra is an important transcriptional factor or master regulator for the pathological outcomes for NASH, gives strong support for their hypothesis. A nice added touch to this paper is that the data shows the statistical variation of all the data within a sample group with a Western blot or graphic as a representative of that data. Although this is a very good and informative manuscript, there are a few weaknesses that have been noted below.

1. Fig 1 shows a comparison between primary human hepatocytes (PHHep) and mouse hepatocytes (AML cells) during starvation in which the authors measured translation rates. The results were similar leading

them to do a proteomic analysis on the AML cells. For relevance in human disease, why not do the same proteomic analysis in the PHHep cells? Was there a technical barrier for doing this?

2. The text describes results for Fig. 4N and 4O as wells as 6I. These figures were not included in the manuscript.

3. Fig. 5A, B, C are all tied together and should be described together in the text as Fig. 5A-C and not separated out.

4. Bottom of page 20 the word “blocked” is used to describe the mode of action of C29. The data suggests that this is not the case, but rather the best word to describe it is “attenuated”.

5. On page 9 and fig. 1J the authors mention that they did a transcription factor analysis to identify Ppargc1a and Esrra as likely transcription factors involved with protein translation. The methodology and the resulting data is not presented in the manuscript. Fig 1J is not very helpful to ascertain what results came from the analysis.