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March 30, 2017

To the Editor:

We would like to express our sincere thanks to the reviewers for a thorough review of our manuscripts, for the excellent suggestions which we feel strengthened the quality of our paper, and for the opportunity to submit a revised manuscript. We are grateful for the comments and have addressed them below.

Response to Comments from Reviewer One:
The manuscript is very well written, easy to follow. The rationale for the study is clear, the study design is adequate and the methodology is clearly described. The results are well presented, and the conclusions are supported by the results. I have only minor comments:
1. Figure 3 is cited in the text (page 10) before Figure 2. 2.
> We have removed the initial reference to figure 3.

2. The quality of Figure 5 B (histology) should be improved. The tissue sections seem damaged those corresponding to AR.
> While there is small damage to this image, the images clearly show expression of AR and FOXM1. However, in response to this concern, as well as point 8 from reviewer 4, we have added magnified images which should make the staining patterns more clear.

Response to Comments from Reviewer Two:
Unfortunately page numbers and lines are not marked, rendering difficult reviewing this MS. This is an interesting study, the paper is well written and results could be potentially relevant. However, there are some problems with the resulst:
1. when describing results shown in Fig. 2C Authors should mention the fact that AR activity is lower in cells transfected with the AR with 65 stretch.
> We have added the requested discussion, along with a citation.

2. results shown in Fig. 3, in general, are not so clear. for intsnace the claimed different expression of FOXM1 is not so apparent in Fig. 3A (also the control p84 show variations). In Fig. 3C, the claimed decrease follwoing siRNA is also not so clear. Overall, the fact that FOXM1 mediates the ability of thiostrepton is not so stronggly evident as claimed (and conclusions in results and discussion should be smoothed in my opinion). Alternatively, the Authors could show better blots.
> We agree that the interpretation is difficult. We could not find a control protein that appeared to be expressed evenly in all cells, despite equal loading of total protein (we tried several actins and GAPDH to no avail). However, the expression of FOXM1 in the cell lines was a very clear binary division. We also agree that the decrease in Fig 3C is not dramatic (and to be clear, it is not due to siRNA, just to thiostrepton treatment), but it is in line with published literature. We have tried to smooth the results and discussion around this figure, which was also something requested by reviewer 4.

3. there are some problems in the description of results of Fig. 5 (some of the panles do not coincide with the description in the results).
> The legend has been revised.

Response to Comments from Reviewer Three:
I believe this ms should be published in its present form. I just have a minor correction/clarification to ask: in the Discussion (third Paragraph, line 6, "...determine exactly how…"), the Authors wanted probably to indicate "thiazole antibiotics", and not "antibodies". Please correct or explain.
> Yes, we meant antibiotics. This has been corrected.

Response to Comments from Reviewer Four:
The article is interesting, well written, and data looks reliable and novel. However, few important points require detailed clarification/additional data.
1. Thiazole antibiotic Thiostrepton has been shown to down-regulate the transcription factor FOXM1 and, thus, the AR inhibition might be also indirect in vivo or in cell based screening. Authors should clearly indicate that fact all over the manuscript and discuss their data in the light of AR indirect inhibition. In other words, authors should discuss the inhibition of AR signaling pathway by means of blockade of potential upstream regulation by transcription factor. The authors should also stress that point in the study, and discuss the possibility of further testing of direct binding of thiazole antibiotics to AR (using other assays, like competitive binding etc).
> We agree with this interpretation and have re-worded multiple sections in response.

2. Established FOXM1 downstream targets (independent from AR signaling) were not assessed.
> We analyzed the transcript level of two know FOXM1 target genes, Ccnd1 and Sox2, in intact vs. thiostrepton treated rat spinal cords by RT-qPCR and found significant down-regulation of both. This has been added to figure 5.

3. Limitations of the study were not discussed.
> We have now included a discussion of the limitations of our study.

4. In the Abstract - I suggest to replace words "… AR activity", "…AR antagonism ..", "…AR selectively in motor neurons.." with " AR conformational transformation…", or "… AR signaling .." or "AR pathway signaling", or " AR indirect blockade mediated by transcription factor FOXM1 etc …" or similar in the meaning phrases. The current stage of abstract is partially misleading/confusing, and gives an impression of direct antagonist-receptor inhibition that in fact was not completely proven.
> Such changes have been made as requested.

5. Methods: "…intensity of FOXM1 expression creating a scale ranging from 0-2"(page 8). It is very short description of the scoring. You have to provide more details and also provide images with highest expression score and with lowest as supplementary data.
> We have expanded the description of the methods and provided the requested images as supplementary data. The images currently included in figure 5 are good representations of high and low expression scores.

6. Figure 3. Statistical analysis of protein expression should be presented. Usually westerns are repeated 3-4-times, and all protein bands (band intensities) mean values are assessed and deviations are calculated and

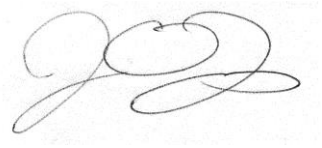
organised as bar graphs or similar for 5 of controls. In Figure legend it is not mentioned how many times the experiments were repeated. P84 expression is not equal, please provide a statement about amount of protein loaded for different cell lines.
> To this figure, we have added quantification to clearly demonstrate our conclusions that thio reduces FOXM1 and reduces nuclear levels of beta catenin. We have performed several replicates of these experiments and show representative versions of each. In the discussion, we state: “Although only one blot is shown in each figure, each of the IP and Western blot experiments was repeated several times, with high reproducibility.” As for P84, please see our response to Reviewer 2, point 2.

7. Figure 4. Nuclear images for the lower panel (DHT + Thios..) look distorted. Why the nuclei are so long and different from the rest of the nuclei in the above images? The cells nuclei look smudged. % of co-localization should be also calculated fro several images (experiment was supposed to be repeated for 3-4- times) and presented as graph with error bars.
> We have replaced the images and provided a graph demonstrating co-localization values. As now mentioned in methods, co-localization values were calculated from three separate experiments.

8. Figure 5. Magnified images should be included as the presented magnification/images give very little information about cellular/intracellular/nuclear localization of the AR. Nuclear co-localization/ or translocation for beta-catenin should be also tested using immunofluorescence/IHC.
> We have now included magnified images to demonstrate subcellular localization of AR and FOXM1. We feel staining for beta-catenin in these samples is beyond the scope of this work.

We greatly appreciate your excellent and detailed feedback, and hope that we have addressed your concerns in a satisfactory manner.

Sincere thanks,



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