Reviewer’s report

Title: Multiple myeloma cells alter the senescence phenotype of bone marrow mesenchymal stromal cells under participation of the DLK1-DIO3 genomic region

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Reviewer: Jose Piruat

Reviewer’s report:

The paper by Berenstein et al. “Multiple myeloma cells alter the senescence phenotype…”, aims to characterize the senescence status of bone marrow-mesenchimal stem cells from MM patients in comparison with those from healthy donors, putting special emphasis on the role of some microRNAs. The experiments are technically well performed and the methodology is appropriate. However, this reviewer has some difficulty to understand the experimental design of the project.

Major Compulsory Revisions

As the authors manifest, “MM-BMMSCs play a critical role in MM tumor growth and survival”. Therefore, it would be interesting to test the effect of MSCs upon KMS12-PE multiple myeloma cell line. In my opinion, this is the real proof-of-concept to be tested, rather than the influence of MM cells on BMMSCs. For instance, the miRNAs analyzed were chosen because they are deregulated in MM cells. Then, why not to look at the expression of these miRNAs in the MM cell line co-cultured with MM-BMMSCs of different origin?. This is an experiment not so difficult to perform, as RNA preps could be easily done from KMS12-PE cells. Likewise, expression of cyclins, p16, p21, etc…could also be tested.

In addition, “the analysis of senescence in MM-BMMSCs displayed similar results as found by others (line 327)”. This deprives of novelty to the initial characterization performed in this work.

Co-culturing experiments: SA-betaGal essay must be done with another cell type (Hela?, fibroblasts? Or even better, B or plasma cells) as controls. Moreover, which is the effect upon HD-BMMSCs?. These experiments are important in order to assess the specificity of the effect.

Minor Essential Revisions

As seen in Figure 1, senescence of Relapsed-MM-BMMSCs is higher than that of newly diagnosed. This point should be discussed.

As gene and miRNA expression data are too disperse, at least Cyclins, p16 and p21 could be tested at protein level (western or IH). Histograms of cell cycle are not informative. The same is true for the co-cultures/transwell experiments.

Discretionary Revisions

What is the purity of isolated BMMSCs?. It would be useful to have this information.
BMMSCs can be removed from axis labels, as it is common to all groups. Some data are difficult to believe, for instance, the statistical significance in figure 4B (methylation status of co-cultured MSCs) should be revisited by a statistician. Experiments with mimics and inhibitors of miR-485 are not informative.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests