Reviewer’s report

Title: Interaction between the BAG1S isoform and HSP70 mediates the stability of anti-apoptotic proteins and the survival of osteosarcoma cells expressing oncogenic MYC

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Reviewer: Sandip K Mishra

Reviewer's report:

In the manuscript entitled "Interaction between the BAG1S isoform and HSP70 mediates the stability of anti-apoptotic proteins and the survival of tumor cells expressing oncogenic MYC", authors have investigated the role of single isoform (BAG1S) of BCL2 Associated Athanogene 1 (BAG1) in its pro-survival activity in the MYC pathway. As BAG1S is found to interact with HSP70 for pro-survival activity, authors have explored the pro-survival effector proteins involved in the pathway. The manuscript is fairly written and the data presented is in line with the hypothesis and conclusions drawn. By addressing comments given below, the authors can improve the overall impact of the manuscript.

Minor comments:

1. Typo error should be rectified:
   line 46 4-hydroxytamoxigen to 4-hydroxytamoxifen
   line 55 TCommon

2. The title of the manuscript is misleading. Authors have used only one human osteosarcoma cell line U2OS in the entire study, however the title suggests the use of patient-derived tumor cells. Instead the title can be:

   "Interaction between the BAG1S isoform and HSP70 mediates the stability of anti-apoptotic proteins and the survival of osteosarcoma U2OS cells expressing oncogenic MYC"

3. Fig 1B, 1F shows the quantified data of FACS analysis. Authors should clarify whether the %Apoptosis in Y-axis is early or late apoptosis or both.

Major comments:

1. In figure 1C, 1G, 2E, 3F authors have shown the image for PARP or Caspase 3 full length and cleaved in separate blots. As shown in the image of the antibody(catalog mentioned in materials
and methods section) used in the study, PARP and cleaved PARP show bands at 116 kD and 89kD respectively, Caspase 3 and cleaved caspase 3 at ~36 and ~17 kD respectively therefore a single blot showing both full length as well as cleaved PARP will be more appropriate and convincing.

2. Under the results section "BAG1 protein required for blocking MYC-driven apoptosis" presented in Fig. 1, authors have taken the advantage of conditional MYC-ER allele for induction of MYC activity upon treatment with the estrogen analog 4-hydroxytamoxifen. A result showing the increased activity/expression of Myc upon 4-OHT treatment will help to correlate the results shown in the figures.

3. Throughout the figures, authors have used the term Myc for 4-OHT treatment that makes the results complicated. As authors have used cell line U2OS expressing Myc-ER allele, use of 4-OHT instead of Myc in the figures will be more illustrative.

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

Yes

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

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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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