

Defending INA-6 Cell Line



User

My paper about with the title "Keep it Together: Describing Myeloma Dissemination in vitro with hMSC-Interacting Subpopulations and their Aggregation/Detachment Dynamics" is under peer review.

I am providing you the abstract to this paper, just so you know what the paper is about:

Multiple myeloma is an accumulation of malignant plasma cells in the bone marrow. How myeloma cells initially disseminate, is still unclear. Mesenchymal stromal cells (MSCs) retain plasma cells in the bone marrow: They provide chemokines and cell adhesion factors. Concurrently, MSCs contribute to the expansion of myeloma cells through growth signals (e.g., IL-6). Therefore, their role in dissemination is ambiguous.

Using time-lapse microscopy, we assessed cell growth and attachment/detachment dynamics of an IL-6-dependent myeloma cell line (INA-6) co-cultured with primary hMSCs. We established separation techniques (V-well adhesion assay, well plate sandwich centrifugation) to isolate and characterize MSC-interacting subpopulations (RNAseq, viability). Results were correlated with survival and gene expression data from myeloma patients (n=536).

INA-6 rapidly adhered and proliferated into homotypic aggregates on dispersed hMSCs. On confluent hMSCs, INA-6 switched to heterotypic interactions, which changed the apoptosis rate of INA-6 in a time-dependent manner. Single myeloma cells detached spontaneously from aggregates but not from hMSCs. Cell division initiated these detachments: daughter cells lost MSC adhesion (nMA-INA6), while mother cells remained MSC-adherent (MA-INA6). nMA-INA6 showed upregulation of proliferation-related genes and superior viability after IL-6 withdrawal. MA-INA6 upregulated adhesion and retention factors (CXCL12), which were also highly expressed in myeloma samples from patients with longer overall and progression-free survival but downregulated in those from relapsed myeloma.

Briefly, INA-6 disseminate in vitro through a cycle of (re)attachment, proliferation, and detachment to and from hMSCs. This cycle involves adhesion factors with potential clinical relevance and implies a role for bone marrow retention in myeloma dissemination.

Now to my question: I am facing critique from a reviewer:

It is questionable if INA-6 cells represent a cell model with a general validity and if experiments carried out using only one cell line may provide information on a general feature of multiple myeloma. Confirming these data using cell lines with different adhesive properties/MSC dependency, parental cell line characterized by IL-6 dependency and the corresponding IL-6 independent variant (i.e. CMA-03 and CMA-03/06 cells) and primary myeloma cells would make results more reliable and generalizable.

I have formulated this response:

- We fully understand that using multiple cell lines is the standard in this field.

- However, we have good reasons to believe that this paper is still worth publishing without adding further cell lines:
 - The list of genes that we associated with patient survival stand independently of used cell lines.
 - The detailed protocols presented in the supplemental provide a solid starting point for other projects studying adhesion.
- Furthermore, we have spent a lot of thought on this topic and came to the conclusion that using more cell lines has, in fact, has major drawbacks for understanding myeloma adhesion:
 - The protocols presented in this study were optimized for one highly adherent cell line (as stated in the discussion). Publishing multiple protocols fine-tuned to the need of other cell lines would add to the complexity of this paper (Our Supplemental already has 29 Pages).
 - Using multiple cell lines could help understand the MSC-Myeloma interface, but will not overcome limitations of in vitro approaches. The most significant example is the capability of in vitro aggregation (Fig. 1C). We can not predict at all if a cell line aggregates both in vitro and in vivo. Yet for this study, in vitro aggregation was highly relevant for observing detachments, which made the introduction of the term "in vitro dissemination" believable in the first place.
 - The adhesive properties of myeloma cell lines are ridiculously and inconceivably diverse, especially in a hMSC-interaction context: E.g. U266 do not adhere strongly to hMSCs; MM.1S adhere to hMSC but also adhere to plastic. Then there is IL-6 independency. It is simply impossible to cover all of that in one publication (although we admit, comparing CMA-03 and CMA-03/06 cells indeed sounds very intriguing.).
 - Facing this diversity and limitations of in vitro data, we highly doubt that any in vitro myeloma data would ever be able to reach "generalizability", but rather introduce artifacts to the field without understanding the sub-cohorts that cause this diversity observed in the adhesive capabilities of myeloma cell lines.
 - Overall, the reviewer has to understand that it makes far more sense to dedicate further publications to other cell lines. And for those, we highly recommend focusing on single cell lines as well, given the ridiculously high variance one can face even within in one cell line. We are convinced that understanding something well is far better than attempting to publish the mean of something we don't understand.

Could you help me with the phrasing? Please make sure to understand the following points before you start writing your version:

- Please understand that my response is structured with a clear distinction between "Our paper is good enough without further cell lines" and "Further cell lines is bad for the field in general", because the second argument challenges a well established standard and should be treated with more carefully. Please structure your version like that as well.
- Make it sound convincing. After all, I am challenging the well-established standard of using multiple cell lines, and it will be hard convincing my peers!
- You see that I have stressed the relevance of certain arguments that are important to me, please maintain that level of importance in these specific arguments.
- Be concise! Skip sentences that are irrelevant to the arguments at hand.



Subject: Response to Reviewer's Comments on Manuscript ID [Your Manuscript ID]
 Dear [Editor's Name],

Thank you for forwarding the reviewer's insightful comments on our manuscript, titled "Keep it Together: Describing Myeloma Dissemination in vitro with hMSC-Interacting Subpopulations and their Aggregation/Detachment Dynamics." We appreciate the opportunity to address the concerns raised, particularly regarding the use of a single cell line in our study.

We acknowledge the reviewer's suggestion of employing multiple cell lines to enhance the generalizability of our findings. While we understand that this is a common practice in the field, we would like to present our rationale for maintaining the focus on the INA-6 cell line in our study.

1. **Independence of Gene Associations:** The gene associations we identified with patient survival stand independently of the specific cell line used. These findings provide valuable insights into the broader context of myeloma dissemination, transcending the limitations of a single cell line.
2. **Robust Protocols for Adhesion Studies:** The detailed protocols provided in the supplementary material offer a solid foundation for investigating adhesion dynamics. We believe this resource can serve as a valuable starting point for researchers studying similar processes with different cell lines.
3. **Optimization Challenges and Protocol Complexity:** Our study optimized protocols for a highly adherent cell line, INA-6, as stated in the discussion. Expanding the study to include multiple cell lines would not only add considerable complexity to the paper (currently 29 pages in the supplementary material) but would also require distinct optimization for each cell line, potentially diluting the clarity of our presented methodologies.
4. **Diversity in Adhesive Properties:** The incredibly diverse adhesive properties of myeloma cell lines, especially in the context of hMSC interactions and IL-6 dependency, present a significant challenge. Attempting to cover this spectrum within a single publication may inadvertently introduce artifacts and compromise the depth of our understanding.
5. **Limitations of In Vitro Approaches:** We emphasize the inherent limitations of in vitro models in capturing the full complexity of myeloma behavior. Our focus on understanding the specific interactions of INA-6 with hMSCs allows for a more in-depth exploration of the phenomena observed, rather than attempting to generalize across a heterogeneous landscape.
6. **Recommendation for Future Publications:** We believe that dedicating future publications to other cell lines would be more productive. We suggest that, even with different cell lines, focusing on a single line of inquiry will yield a more comprehensive understanding than attempting to publish an average of phenomena that may be inherently diverse.

In conclusion, we appreciate the importance of using multiple cell lines in research, but we firmly believe that, in the context of our study, the depth of understanding achieved with the INA-6 cell line outweighs the challenges associated with introducing additional cell lines. We hope that our rationale aligns with the objectives of the journal and would be happy to make further revisions if deemed necessary.

Thank you for your consideration.

Sincerely,

[Your Full Name]

[Your Affiliation]

[Your Contact Information]

ChatGPT kann Fehler machen. Überprüfe wichtige Informationen.