

Cell Line Validity Defense



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 made 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.



Subject: Response to Reviewer's Comments on Manuscript [Your Manuscript Title]

Dear [Reviewer's Name],

Thank you for taking the time to review our manuscript titled "Keep it Together: Describing Myeloma Dissemination in vitro with hMSC-Interacting Subpopulations and their Aggregation/Detachment Dynamics." We appreciate your valuable feedback and the opportunity to address your concerns.

We acknowledge the established practice in the field of utilizing multiple cell lines in research studies, and we understand the importance of generalizability. However, we would like to present our perspective on why we believe our paper remains valuable and impactful without incorporating additional cell lines.

Firstly, the genes associated with patient survival, a significant aspect of our study, stand independently of the specific cell line used. These findings provide insights that extend beyond the limitations of a single cell line and contribute to the broader understanding of myeloma dissemination.

Moreover, the detailed protocols we have provided in the supplemental materials offer a robust foundation for future studies focusing on adhesion. These protocols were meticulously developed and optimized for the highly adherent INA-6 cell line, ensuring the reproducibility of our results. The inclusion of multiple cell lines would introduce complexity and potentially dilute the clarity of our methodology.

While we acknowledge the diversity in adhesive properties among myeloma cell lines, attempting to cover this vast variability within a single publication poses challenges. Our supplemental materials already span 29 pages, and expanding further to accommodate multiple cell lines could compromise the comprehensibility of the presented data.

Importantly, we have carefully considered the limitations of in vitro approaches, especially in understanding the MSC-myeloma interface. The introduction of the term "in vitro dissemination" was based on the relevance of in vitro aggregation, a phenomenon observed in our study. Incorporating additional cell lines might not address the inherent limitations of in vitro data and could potentially introduce artifacts without a deeper understanding of the underlying sub-cohorts responsible for the observed diversity in adhesive capabilities.

We would like to emphasize that the highly diverse adhesive properties of myeloma cell lines, coupled with factors such as IL-6 dependency and in vitro aggregation, make it practically challenging to encompass all aspects within a single publication. While the use of multiple cell lines is a common practice, we believe that in this specific study, a focused approach on a single cell line allows for a more in-depth exploration and understanding of the phenomena under investigation.

In conclusion, we respectfully assert that our study offers valuable insights into myeloma dissemination, and the limitations associated with in vitro data are inherent to the field. Dedication to understanding specific aspects thoroughly is, in our view, more advantageous than attempting to average results from diverse and potentially incomparable cell lines. We hope our rationale for maintaining the current scope of our study is compelling and aligns with the unique contributions our work brings to the field.

Thank you for considering our response, and we look forward to your further feedback.

Sincerely,

[Your Full Name]

[Your Affiliation]

[Your Contact Information]

ChatGPT kann Fehler machen. Überprüfe wichtige Informationen.