

Myeloma Dissemination: INA-6 & hMSC Interaction



I am writing a paper about an invitro model of myeloma dissemination. Please see these results and give your best to understand them. Afterwards, I'm going to tell you what I want in detail:

INA-6 Cells Saturate hMSC-Interaction to Proliferate into Aggregates

To analyze whether INA-6 cells can adhere to hMSC despite their heterogeneous nature, we attempted to saturate hMSCs with INA-6. At an hMSC:INA-6-ratio of 1:4, $93\% \pm 6\%$ of single hMSCs were occupied by one or more INA-6 cells within 24 h after INA-6 addition, reaching up to 98% after 48 h (Fig. 1A, B). Therefore, most hMSCs could provide an interaction surface for INA-6 cells. INA-6 forms homotypic aggregates when cultured alone, which has also been described in some cases of freshly isolated myeloma samples (up to 100 cells after 6 h) (33,34). However, when culturing INA-6 with hMSCs at a 1:1 ratio for 24 h, most INA-6 cells formed small aggregates (size 1-5 cells), all of which distributed over $52\% \pm 2\%$ of all hMSCs (Fig. 1A, B). Correspondingly, INA-6 aggregation did not occur when grown on confluent hMSC. Aggregation occurred only when heterotypic interactions were limited to 0.2 hMSCs per INA-6 cell (Fig. 1C). We concluded that INA6 cells prioritize heterotypic over homotypic interactions.

To monitor how such aggregates were formed, we conducted live-cell imaging of hMSC INA-6 co-cultures over 63 h. We observed that INA-6 cells kept adhering long after cytokinesis, which accounted for $55\% \pm 12\%$ of all homotypic interactions between 13 and 26 h, increasing to $>75\%$ for the rest of the co-culture (Fig. 1D). Therefore, homotypic INA-6 aggregates were mostly established by cell division.

Apoptosis of INA-6 Depends on Ratio Between Heterotypic and Homotypic Interaction

Direct interaction with hMSCs is known to increase the survival of myeloma cells via NF- κ B signaling (15). However, little is known about how aggregation affects myeloma cell survival during hMSC-interaction. Hence, we measured cell viability (ATP) and apoptosis rates of INA-6 growing as homotypic aggregates and those growing in heterotypic interaction with hMSCs (Fig. 1E). To display hMSC-adhesion effects, all cultures incubated in hMSC-conditioned medium and were normalized to INA-6 cultured without direct hMSC contact (Fig. 1E, left).

INA-6 viability (ATP) was not affected by direct adhesion of hMSCs at any density. However, apoptosis decreased with time [$F(2, 6) = 23.29$, $p\text{-unc} = 1.49\text{e-}03$, Two-factor RM-ANOVA], which was enhanced at higher MSC density [interaction between MSC density and time: $F(4, 12) = 6.98$, $p\text{-unc} = 3.83\text{e-}3$]. For example, 24 h of adhesion to confluent MSCs increased by 1.46 ± 0.37 fold, while culturing on dispersed hMSCs (ratio 1:1) did not change apoptosis (1.01 ± 0.26).

We presumed that sensitive apoptotic cells might be lost when harvesting INA-6 cells from hMSCs. Hence, we also measured survival parameters in the co-culture and hMSC/INA-6 cultured alone (Fig. 1E, right). We defined MSC interaction effects when the survival measured in the co-culture differed from the sum of signals measured from INA-6 and hMSCs alone. RM-ANOVA confirmed that adherence to confluent MSCs increased apoptosis of INA-6 after 24 h of adhesion and decreased after 72 h [interaction between MSC density and time: $F(2, 4) = 26.86$, $p\text{-unc} = 4.80\text{e-}03$, Two-factor RM-ANOVA], whereas INA-6 cells was unaffected when grown on dispersed hMSCs. In summary, the growth conformation of INA-6 cells, measured as the ratio between homotypic

aggregation and heterotypic MSC interactions, affected apoptosis in INA-6 cells.

Single INA-6 Cells Detach Spontaneously from Aggregates of Critical Size

Time-lapse acquisitions showed that $26\% \pm 8\%$ of observed INA-6-aggregates growing on top of single hMSCs spontaneously lost INA-6 cells (Fig. 2A, B; Vid. S1). Because all detached cells moved in a similar direction, we assumed that these cells were caught in convective streams that arose from the temperature gradients of the incubation chamber. The frequency of detachment increased after 52 h. From this time on, INA-6 cells detached mostly from other INA-6 cells (Fig. 2B), showing that homotypic interactions involved weaker adhesive forces than heterotypic interactions. Furthermore, most aggregates that lost INA-6 cells were categorized as large ($> \sim 30$ cells) (Fig. 2C). Since ~ 1020 INA-6 cells already fully covered a single hMSC, we suggest that myeloma cell detachment depended not only on hMSC saturation but also required a minimum aggregate size. However, INA6 detached mostly as single cells, independent of aggregate size categories [F(2, 6)=4.68, p-unc=.059, Two-factor RM-ANOVA] (Fig. 2D).

We concluded that the mechanism of detachment was similar across each stage of aggregate proliferation, whereas the detachment frequency increased with aggregate size.

Cell Division Generates a Daughter Cell Detached from hMSC

We suspected that INA-6 cells could detach from hMSCs immediately after cell division, since we observed that adherent INA-6 cells could generate daughter cells that “roll over” the adherent mother cell (Fig. 3A; Vid. S2). We recorded and categorized the movement of INA-6 daughter cells on confluent hMSCs after cell division and found that half of all INA-6 divisions yielded two daughter cells that did not move, indicating hMSC-adherence (Fig. 3B, C; Vid. S3). The other half of the division events generated one hMSC-adhering (MA) cell and one non-hMSC-adhering (nMA) cell that rolled around the MA cell for a median time of 2.5 h post division (Q1=1.00 h, Q3=6.25 h) until it stopped and re-adhered to the hMSC monolayer (Fig. 3D; Vid. S2, S4). Therefore, cell division creates a time window during which one daughter cell can detach.

To validate that cell division reduced adhesion, we measured both the size and cell cycle profile of the nMA and MA populations using an enhanced V-Well Assay (method described in Fig. 3E, S1, S2). We compared the results with those of INA-6 cells that were fully arrested at mitosis and released immediately before adding them to an hMSC monolayer, rendering them more likely to divide while adhering to hMSC. Mitotic arrest significantly increased the number of nMA cells and decreased the number of MA cells (Fig. 3F). Furthermore, the nMA population contained significantly more cells cycling in the G0/G1 phase than the MA population, both in synchronously and asynchronously cycling INA-6 (Fig. 3G, S3B). This shows that the number of nMA INA-6 cells could be increased by increasing the frequency of cell division.

Together, we showed that INA-6 could detach from aggregates by generating one (temporarily) detached daughter cell after cell division, presenting a potential contributor to the initiation of dissemination.

Well Plate Sandwich Centrifugation (WPSC) Separates hMSC-Interacting INA-6 Subpopulations

To separate nMA and MA cells for further analysis, we developed the method “Well Plate Sandwich Centrifugation” (WPSC, described in Fig. 4A). Again, we aimed to display effects of direct interaction. Hence, we seeded co-cultures in hMSC-conditioned medium (CM) from the respective donors and compared results to INA-6 incubating in CM without hMSCs.

We microscopically tracked the numbers of both nMA and MA INA-6 after each separation and centrifugation step during WPSC. Both populations were sufficiently separated after the third centrifugation, whereas CM-treated INA-6 cells alone required only one centrifugation (Fig. 4B). Hence, WPSC yield cell numbers that are sufficiently high for further analysis.

RNAseq of non-MSC-Adhering and MSC-Adhering Subpopulations

To characterize the subpopulations separated by WPSC, we performed RNAseq. We identified 1291 differentially expressed genes between nMA vs. CM, 484 between MA vs. CM, and 195 between MA vs. nMA. We validated RNAseq and found that the differential expression of 18 genes correlated with those measured with qPCR for each pairwise comparison (Fig. 4C-E, S5): nMA vs. CM [$p(16) = .803$, $p = 6.09e-5$], MA vs. CM [$p(16) = .827$, $p = 2.30e-5$], and MA vs. nMA cells [$p(16) = .746$, $p = 3.74e-4$] (Spearman's rank correlation). One of the 18 genes (MUC1) measured by qPCR showed a mean expression opposite to that obtained by RNAseq (nMA vs. CM), although the difference was insignificant (Fig. 4C). For nMA vs. CM, the expression difference measured by qPCR was significant for only two of 11 genes (DKK1 and OPG), while the other genes (BCL6, BMP4, BTG2, IL10RB, IL24, Notch2, TNFRSF1A, TRAF5) only confirmed the tendency measured by RNAseq (Fig. 4C-E). For MA vs. CM, qPCR validated the significant upregulation of seven genes (TGM2, DCN, LOX, MMP14, MMP2, CXCL12, CXCL8), whereas the downregulation of BMP4 was insignificant.

Non-MSC-Adhering INA-6 and MSC-Adhering INA-6 Have Distinct Expression Patterns of Proliferation or Adhesion, Respectively

To functionally characterize the unique transcriptional patterns in nMA-INA6 and MA-INA6, we generated lists of genes that were differentially expressed vs. the other two subpopulations [termed nMA vs. (MA & CM) and MA vs. (nMA & CM)]. We then performed functional enrichment analysis and displayed the enriched terms as ontology clusters (Fig. 5A). nMA-INA6 upregulated genes enriched with loosely connected term clusters associated with proliferation (e.g., "positive regulation of cell cycle"). MA-INA6 upregulated genes enriched with tightly connected term clusters related to cell adhesion and the production of ECM factors (e.g. "cell-substrate adhesion"). Similar ontology terms were enriched in gene lists from pairwise comparisons (nMA vs. CM, MA vs. CM, MA vs. nMA) (Fig. 5B). In particular, nMA vs. CM (but not MA vs. CM) upregulated genes that were enriched with G1/S transition, showing that WPSC isolated nMA daughter cells after cell division.

To check for similarities between lists of differentially expressed genes from hMSC-interacting subpopulations, we performed enrichment analysis on gene lists from the overlaps ("∩") between all pairwise comparisons (Fig. 5B, S6), and showed the extent of these overlaps in circos plots (Fig. 5C). The overlap between MA vs. CM and nMA vs. CM showed neither enrichment with proliferation- nor adhesion-related terms but with apoptosis-related terms. A direct comparison of MSC-interacting subpopulations (MA vs. nMA) showed a major overlap with MA vs. CM (Fig. 5C, middle). This overlap was enriched with terms related to adhesion but not proliferation. Hence, MA-INA6 and nMA-INA6 mostly differed in adhesion gene expression.

To assess whether nMA-INA6 and MA-INA6 are regulated by separate transcription factors, we examined the enrichment of curated regulatory networks from the TRRUST database (Fig. 5B bottom). All lists were enriched for p53 regulation. Regulation by E2F1 was found only in genes upregulated in nMA vs. CM and downregulated in MA vs. nMA. Genelists involving MA-INA6 were enriched in regulation by subunits of NF- κ B (NFKB1/p105 and RELA/p65) and factors of the immediate early response (SRF, JUN). Accordingly, NF- κ B and JUN are known to mediate cell adhesion in multiple myeloma and B-cell lymphoma, respectively (35,36). Furthermore, RELA/p65 has been reported to antagonize p53, implying a potential switch from the regulation seen in nMA-INA6 (37,38).

Taken together, MSC-interacting subpopulations show unique regulatory patterns, focusing on either proliferation or adhesion.

nMA-INA6 and MA-INA6 Show Increased Apoptosis Signaling Mediated by ER-Stress, p53 and Death Domain Receptors

We observed that apoptosis was increased in INA-6 growing on confluent hMSCs compared to CM-INA6 after 24 h of co-culture (Fig. 1D). Since this setup was similar to that used to separate

hMSC-interacting subpopulations using WPSC, we looked for enrichment of apoptosis-related terms (Fig. 5B).

“Regulation of cellular response to stress” and “intrinsic apoptotic signaling pathway (in response to ER-stress)” are terms that were enriched in nMA vs. CM, MA vs. CM and their overlap. We also found specific stressors for either nMA-INA6 (“intrinsic apoptotic signaling pathway by p53 class mediator”) or MA-INA6 (“extrinsic apoptotic signaling pathway via death domain receptor”). Therefore, apoptosis may be driven by ER stress in both nMA-INA6 and MA-INA6, but also by individual pathways, such as p53 and death domain receptors, respectively.

nMA-INA6 and MA-INA6 Regulate Genes Associated with Bone Loss

Myeloma cells cause bone loss by degradation and dysregulation of bone turnover through DKK1 and OPG (39–41). RNAseq of hMSC-interacting subpopulations showed enrichment with the functional term “ossification” (Fig. S6), as well as the regulation of MMP2, MMP14, DKK1, and OPG. Validation by qPCR (Fig. 4C, D) showed MA-INA6 significantly upregulating both MMP14 and MMP2 compared to either nMA-INA6 or CM-INA6. The expression of DKK1, however, was upregulated significantly in nMA-INA6 (and not significantly upregulated in MA-INA6), while OPG was significantly downregulated only in nMA-INA6.

Together, hMSC-interacting subpopulations might contribute to bone loss through different mechanisms: MA-INA6 by expressing matrix-metalloproteinases; nMA-cells by paracrine signaling.

MA-INA6 Upregulate Collagen and Chemokines Associated with Bone Marrow Retention

Retention of myeloma cells within the bone marrow is mediated both by adhesion to the ECM (e.g., collagen VI) and secretion of chemokines (CXCL8, CXCL12) (6,9). This directly counteracts dissemination, which we expected to be a hallmark of MA-INA6. RNAseq of hMSC-interacting subpopulations showed that genes upregulated in MA-INA6 were enriched with collagen biosynthesis and modifying enzymes, as well as chemotaxis and chemotaxis-related terms (Fig. 5B). Using qPCR, we validated the upregulation of collagen crosslinkers (LOX and TGM2), collagen-binding DCN and chemokines (CXCL8 and CXCL12) in MA-INA6 compared to both nMA-INA6 and CM-INA6 (Fig. 4D). Therefore, MA-INA6 could provide both an adhesive surface and soluble signals for the retention of malignant plasma cells in the bone marrow.

nMA-INA6 Show Highest Viability During IL-6 Withdrawal

Although RNAseq did not show IL-6 induction in any WPSC-isolated subpopulation, nMA-INA-6 upregulated IGF-1 135%-fold [RNAseq, nMA vs. (MA & CM)], which implies an increased autonomy from IL-6 and better survival outside the bone marrow after dissemination (42).

To test the autonomy of hMSC-interacting INA-6 subpopulations, we isolated them by WPSC after 24 h and 48 h of co-culture, sub-cultured them for 48 h under IL-6 withdrawal and measured both viability and apoptosis (Fig. 5D). Among the subpopulations, nMA-INA6 was the most viable.

Compared to MA-INA6, nMA-INA6 increased viability by 8 or 4 fold when co-cultured for 24 h or 48 h, respectively [Hedges g of Log10(Fold Change) = 2.31 or 0.82]. However, the difference was no longer significant for 48 h of co-culturing, probably because nMA-INA6 adhered to the hMSC layer (turning into MA-INA6) during prolonged co-culture, which could also explain why the viability MA-INA6 cell sub-cultures increased with prolonged co-culture. Nevertheless, nMA-INA6 did not achieve the same viability as INA-6 cells cultured with IL6.

Despite the differences in viability, sub-cultures of hMSC-interacting subpopulations did not show any differences Caspase 3/7 activity when co-cultured for 48 h (Fig. 5D, right).

Overall, nMA-INA6 had the best chances of surviving IL-6 withdrawal among the hMSC-interacting subpopulations.

Genes Upregulated by MA-INA6 are Associated with an Improved Disease Prognosis

To relate the adhesion of MA-INA6 observed in vitro to the progression of multiple myeloma, we

assessed patient survival [n = 535, Seckinger et al. 2018 (29,30)] depending on the expression level of 101 genes, which were upregulated in MA vs. (nMA & CM) and are part of the ontology terms "Extracellular matrix organization", "ECM proteoglycans", "cell-substrate adhesion" and "negative regulation of cell-substrate adhesion" (Fig. 6A, Tab. S2). As a reference, we generated a list of 173 cell cycle-related genes that were upregulated by nMA-INA6 vs. (MA-INA6 & CM-INA6). As expected, longer patient survival was associated with low expression of the majority of cell cycle genes [71 or 68 genes for progression-free survival (PFS) or overall survival (OS)]. Only a few cell cycle genes (two for PFS and seven for OS) were associated with survival when highly expressed. Intriguingly, adhesion genes showed an inverse pattern: a large group of adhesion genes (24 for PFS and 26 for OS) was significantly associated with improved survival when highly expressed, while only a few genes (two for PFS and four for OS) improved survival when expressed at low levels. We conclude that the myeloma-dependent expression of adhesion factors determined in our in vitro study correlates with improved patient survival.

Expression of Adhesion- or Retention-related genes (CXCL12, DCN and TGM2) is Decreased During Progression of Multiple Myeloma

To examine how disease stages affect the adhesion and bone marrow retention of myeloma cells in vitro, we displayed the expression of CXCL12 in healthy plasma cells (BMPC) cohorts of patients at different disease stages and in myeloma cell lines (HMCL) [described in Seckinger et al. 2018 (29)] (Fig. 6C). We also included DCN and TGM2 since both are suggested to inhibit metastasis in different cancers by promoting cell-matrix interactions (11,43). In accordance with independent reports (10,44), high expression of CXCL12 and DCN by myeloma cells was associated with improved overall survival (adj. p = .009 and .008, respectively) (Fig. 6B).

CXCL12 is expressed by BMPCs (median = 219 normalized counts), but its expression levels are significantly lower from MGUS to relapsed multiple myeloma (MMR) (median = 9 normalized counts in MMR and absent expression in most HMCL). DCN (but not TGM2) was weakly expressed in BMPCs (Q1=0.7, Q3=3.7 normalized counts), whereas TGM2 was weakly expressed only in patients with monoclonal gammopathy of undetermined significance (MGUS) (Q1=0.4, Q3=4.1 normalized counts). The median and upper quartiles of both DCN- and TGM2 decreased continuously after each stage, ending at Q3=0.9 and Q3=0.6, respectively, in MMR. 49 of the 101 adhesion genes (Fig. 6A) followed a similar pattern of continuous downregulation in the advanced stages of multiple myeloma (Fig. S7, S8), of which 19 genes were associated with longer PFS when highly expressed. The other 52 (of 101) adhesion genes that weren't downregulated across disease progression (or were expressed at a level too low to make that categorization) contained only five genes that were associated with longer PFS at high expression (Tab. 1, Tab. S2).

Together, the expression of adhesion or bone marrow retention-related markers (CXCL12, DCN, and TGM2) is reduced or lost at advanced stages of multiple myeloma, which could enhance dissemination and imply increased independence from the BM microenvironment.



Your detailed results provide a comprehensive overview of the interactions between INA-6 myeloma cells and hMSCs, shedding light on aspects like adhesion, aggregation, apoptosis, detachment, cell division, and the impact of these interactions on gene expression. Here's a summary and understanding of the key findings:

1. Adhesion and Aggregation:

- At an hMSC ratio of 1:4, most hMSCs provided an interaction surface for INA-6 cells.

- INA-6 cells formed small aggregates (1-5 cells) when cultured with hMSCs, and this aggregation was influenced by the density of hMSCs.

2. Apoptosis:

- Apoptosis of INA-6 cells depended on the ratio between heterotypic and homotypic interactions.
- Adhesion to confluent MSCs increased apoptosis after 24 h and decreased after 72 h, suggesting a dynamic regulation of apoptosis during interaction.

3. Detachment and Cell Division:

- Spontaneous detachment of INA-6 cells from aggregates occurred, especially from larger aggregates.
- Cell division created a time window during which one daughter cell could detach, emphasizing the potential contribution to dissemination.

4. Well Plate Sandwich Centrifugation (WPSC):

- A novel method, WPSC, successfully separated non-MSC-adhering (nMA) and MSC-adhering (MA) subpopulations for further analysis.

5. RNAseq Analysis:

- Distinct gene expression patterns were identified between nMA and MA subpopulations.
- Functional enrichment analysis revealed that nMA-INA6 was associated with proliferation, while MA-INA6 was associated with cell adhesion and ECM factors.

6. Functional Characterization:

- MA-INA6 exhibited upregulation of genes associated with bone loss, MMPs, and chemokines related to bone marrow retention.
- nMA-INA6 upregulated IGF-1, suggesting increased autonomy from IL-6 and better survival outside the bone marrow.

7. Patient Survival Analysis:

- Expression of adhesion-related genes in MA-INA6 correlated with improved patient survival compared to cell cycle-related genes.
- Adhesion-related genes were associated with longer progression-free and overall survival.

8. Disease Progression and Gene Expression:

- The expression of adhesion-related markers, including CXCL12, DCN, and TGM2, decreased in advanced stages of multiple myeloma, potentially contributing to dissemination.

In summary, your findings suggest that the interactions between INA-6 cells and hMSCs play a crucial role in myeloma dissemination, influencing cell behavior, gene expression, and potential clinical outcomes. The detailed molecular insights provided by your study have implications for understanding the mechanisms underlying myeloma progression and may contribute to the development of targeted therapies.



Alright! thanks! During peer-review, a reviewer said that it is extremely difficult to follow! The results are already somewhat revised to tackle that, but I'm gonna need your help later. But first I want you to take a look at my abstract. Do you have any feedback on it? Do you think it's hard to follow? I also would like to ask you to shorten the abstract, since its current wordcount is 265, but the upper limit is 250, so it needs to be 15 words shorter. Here's the abstract:

Abstract

Multiple myeloma is an accumulation of malignant plasma cells in the bone marrow coupled with aggressive dissemination. It is still unclear how myeloma cells initially disseminate. The bone

marrow microenvironment stimulates myeloma cell expansion (by e.g., IL-6), but also retains cells through chemokines (e.g. CXCL12) and cell adhesion factors. Hence, we hypothesized that an imbalance between cell division and retention drives dissemination.

Here, we propose an in vitro model utilizing human bone marrow derived mesenchymal stromal cells (hMSCs) co-cultured with the myeloma cell line INA-6. Using time-lapse microscopy, we assessed cell growth and attachment/detachment dynamics in detail. 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 showed low expression 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 that could represent a bone marrow retentive phenotype with potential clinical relevance.



Your abstract provides a clear overview of your study, and the information is well-organized. However, it is slightly longer than the specified word limit. Here's a revised version to meet the 250-word limit:

Revised Abstract:

Multiple myeloma involves aggressive dissemination of malignant plasma cells within the bone marrow, yet the initial steps of dissemination remain unclear. The bone marrow microenvironment influences myeloma cell expansion (e.g., IL-6), retention (chemokines like CXCL12), and cell adhesion. We hypothesized that an imbalance between cell division and retention drives dissemination.

We present an in vitro model using human bone marrow-derived mesenchymal stromal cells (hMSCs) co-cultured with the myeloma cell line INA-6. Time-lapse microscopy reveals detailed cell growth and attachment/detachment dynamics. Separation techniques (V-well adhesion assay, well plate sandwich centrifugation) isolate MSC-interacting subpopulations (RNAseq, viability), correlated with patient survival and gene expression data (n=536).

INA-6 adheres and proliferates rapidly into homotypic aggregates on dispersed hMSCs. On confluent hMSCs, INA-6 switches to heterotypic interactions, influencing apoptosis in a time-dependent manner. Single myeloma cells spontaneously detach from aggregates but not from hMSCs. Cell division initiates detachments: daughter cells (nMA-INA6) lose MSC adhesion, while mother cells (MA-INA6) remain MSC-adherent. nMA-INA6 upregulates proliferation-related genes and exhibits superior viability after IL-6 withdrawal. MA-INA6 upregulates adhesion and retention factors (e.g., CXCL12). These factors are highly expressed in myeloma samples from patients with longer survival, contrasting low expression in relapsed myeloma.

In summary, INA-6 disseminates in vitro through a cycle of (re)attachment, proliferation, and detachment involving adhesion factors, representing a potential bone marrow retentive phenotype

with clinical relevance.



ok, that was very helpful! Here's the finished abstract!

Multiple myeloma involves aggressive dissemination of malignant plasma cells within the bone marrow, yet the initial steps of dissemination remain unclear. The bone marrow microenvironment stimulates myeloma cell expansion (by e.g., IL-6), but also retains cells through chemokines (e.g. CXCL12) and cell adhesion factors. Hence, we hypothesized that an imbalance between cell division and retention drives dissemination.

We present an in vitro model using human bone marrow derived mesenchymal stromal cells (hMSCs) co-cultured with the myeloma cell line INA-6. Time-lapse microscopy reveals detailed cell growth and attachment/detachment dynamics. Separation techniques (V-well adhesion assay, well plate sandwich centrifugation) were established to isolate MSC-interacting subpopulations. Subpopulations were characterized (RNAseq, Cell survival) and results were correlated with patient overall and progression-free survival and gene expression data (n=536).

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Briefly, INA-6 disseminate in vitro through a cycle of (re)attachment, proliferation, and detachment to and from hMSCs. This cycle involves adhesion factors that could represent a bone marrow retentive phenotype with potential clinical relevance.



Your revised abstract is well-crafted, concise, and effectively conveys the key findings of your study. It maintains clarity while adhering to the specified word limit. The structure is logical, and the language is precise, making it easier for readers to grasp the essential aspects of your research. Well done! If you have any further questions or need additional assistance, feel free to ask.



ok thanks!

Now I'd like to ask you for feedback on the results section that I showed you earlier. Do you have feedback to any specific parts of it?



Certainly! The results section you provided is comprehensive, but I'll focus on a few aspects that might benefit from clarification or adjustment:

1. Clarity of Findings:

- Ensure that the language used is clear and straightforward, especially when describing complex interactions and processes. Consider simplifying sentences for easier comprehension.

2. Organization:

- Check the logical flow of information. Make sure that the results are presented in a sequence that follows the natural progression of your experiments and analyses.

3. Use of Abbreviations:

- Double-check the use of abbreviations. While some, like INA-6 and hMSCs, may be common in your field, make sure less common abbreviations are explained or introduced properly.

4. Graphs and Figures:

- If applicable, consider including graphs or figures to visually represent certain data points. Visual aids can enhance the reader's understanding and make the information more accessible.

5. Statistical Significance:

- Clearly indicate the statistical significance of your findings. If possible, consider using p-values or other statistical measures to support your statements about the results.

6. Integration of Figures and Text:

- If you have any figures or images related to your results, make sure that they are appropriately referred to and discussed in the text. Explain the key insights derived from these figures.

7. Conclusion of Results:

- Summarize the key findings at the end of the results section. This can serve as a transition to the discussion section and help the reader understand the significance of the results.

Remember that clarity is key, and the results section should provide a clear and accurate representation of your findings. If you have specific paragraphs or parts you'd like more detailed feedback on, feel free to point them out!



Could you please provide specific sections in my results that could be improved together with a better version of that section?



Certainly! Let's focus on improving the clarity and structure of specific sections in your results. I'll provide suggestions for refinement:

Original Section:

Section: Apoptosis of INA-6 Depends on Ratio Between Heterotypic and Homotypic Interaction

Original Text:

"Direct interaction with hMSCs is known to increase the survival of myeloma cells via NF- κ B signaling (15). However, little is known about how aggregation affects myeloma cell survival during hMSC-interaction. Hence, we measured cell viability (ATP) and apoptosis rates of INA-6 growing as homotypic aggregates and those growing in heterotypic interaction with

hMSCs (Fig. 1E). To display hMSC-adhesion effects, all cultures incubated in hMSC-conditioned medium and were normalized to INA-6 cultured without direct hMSC contact (Fig. 1E, left)."

Improved Version:

Section: Apoptosis of INA-6 Depends on Ratio Between Heterotypic and Homotypic Interaction

Improved Text:

"While direct interaction with hMSCs has been shown to enhance myeloma cell survival through NF- κ B signaling (15), the impact of aggregation on myeloma cell viability during hMSC interaction remains unclear. To address this, we measured cell viability (ATP) and apoptosis rates of INA-6 cells growing as homotypic aggregates and in heterotypic interaction with hMSCs (Fig. 1E). For clarity on hMSC-adhesion effects, all cultures were incubated in hMSC-conditioned medium and normalized to INA-6 cultured without direct hMSC contact (Fig. 1E, left)."

Original Section:

Section: Single INA-6 Cells Detach Spontaneously from Aggregates of Critical Size

Original Text:

"Time-lapse acquisitions showed that $26\% \pm 8\%$ of observed INA-6-aggregates growing on top of single hMSCs spontaneously lost INA-6 cells (Fig. 2A, B; Vid. S1). Because all detached cells moved in a similar direction, we assumed that these cells were caught in convective streams that arose from the temperature gradients of the incubation chamber. The frequency of detachment increased after 52 h. From this time on, INA-6 cells detached mostly from other INA-6 cells (Fig. 2B), showing that homotypic interactions involved weaker adhesive forces than heterotypic interactions."

Improved Version:

Section: Single INA-6 Cells Detach Spontaneously from Aggregates of Critical Size

Improved Text:

"In our time-lapse observations, $26\% \pm 8\%$ of INA-6 aggregates situated on single hMSCs exhibited spontaneous detachment (Fig. 2A, B; Vid. S1). The consistent directionality of detached cells led us to infer their movement within convective streams generated by temperature gradients in the incubation chamber. Notably, the detachment frequency surged after 52 h, predominantly involving INA-6 cells detaching from other INA-6 cells (Fig. 2B), suggesting weaker adhesive forces in homotypic interactions compared to heterotypic interactions."

Feel free to let me know if you'd like improvements in specific sections or if you have other parts you'd like assistance with!



that's a good start, I'd love to see your feedback on every section of my results!



Absolutely, let's work through the entire results section with improvements. I'll provide suggestions for each subsection:

1. INA-6 Cells Saturate hMSC-Interaction to Proliferate into Aggregates

Original Text:

"To analyze whether INA-6 cells can adhere to hMSC despite their heterogeneous nature, we attempted to saturate hMSCs with INA-6. At an hMSC of 1:4, 93% \pm 6% of single hMSCs were occupied by one or more INA-6 cells within 24 h after INA-6 addition, reaching up to 98% after 48 h (Fig. 1A, B). Therefore, most hMSCs could provide an interaction surface for INA-6 cells."

Improved Text:

"To investigate the adhesive capabilities of INA-6 cells to hMSCs, we aimed to saturate hMSCs with INA-6. Achieving an hMSC ratio of 1:4 resulted in the occupation of 93% \pm 6% of single hMSCs by one or more INA-6 cells within 24 h after INA-6 addition, escalating to 98% after 48 h (Fig. 1A, B). These findings underscore the robust interaction surface INA-6 cells can establish on hMSCs."

2. INA-6 Forms Homotypic Aggregates in hMSC Co-Culture

Original Text:

"INA-6 forms homotypic aggregates when cultured alone, which has also been described in some cases of freshly isolated myeloma samples (up to 100 cells after 6 h) (33,34). However, when culturing INA-6 with hMSCs at a 1:1 ratio for 24 h, most INA-6 cells formed small aggregates (size 1-5 cells), all of which distributed over 52% \pm 2% of all hMSCs (Fig. 1A, B). Correspondingly, INA-6 aggregation did not occur when grown on confluent hMSC. Aggregation occurred only when heterotypic interactions were limited to 0.2 hMSCs per INA-6 cell (Fig. 1C). We concluded that INA6 cells prioritize heterotypic over homotypic interactions."

Improved Text:

"While INA-6 exhibits homotypic aggregation when cultured in isolation, a phenomenon observed in freshly isolated myeloma samples (up to 100 cells after 6 h) (33,34), the dynamics shift when co-cultured with hMSCs at a 1:1 ratio for 24 h. In this scenario, most INA-6 cells form small aggregates (size 1-5 cells), distributed over 52% \pm 2% of all hMSCs (Fig. 1A, B). Intriguingly, INA-6 aggregation is notably absent when grown on confluent hMSCs, occurring only when heterotypic interactions are

limited to 0.2 hMSCs per INA-6 cell (Fig. 1C). These findings suggest a prioritization of heterotypic over homotypic interactions by INA6 cells."

I'll continue with the next sections. Let me know if you have specific preferences or if there's anything you'd like to highlight!



please continue with the next sections!



3. Live-Cell Imaging of INA-6/hMSC Co-Cultures

Original Text:

"To monitor how such aggregates were formed, we conducted live-cell imaging of hMSC INA-6 co-cultures over 63 h. We observed that INA-6 cells kept adhering long after cytokinesis, which accounted for $55\% \pm 12\%$ of all homotypic interactions between 13 and 26 h, increasing to $>75\%$ for the rest of the co-culture (Fig. 1D). Therefore, homotypic INA-6 aggregates were mostly established by cell division."

Improved Text:

"To gain insights into aggregate formation dynamics, live-cell imaging of hMSC INA-6 co-cultures was conducted over 63 h. A notable observation was that INA-6 cells continued to adhere long after cytokinesis, constituting $55\% \pm 12\%$ of all homotypic interactions between 13 and 26 h, with a subsequent increase to $>75\%$ for the remainder of the co-culture (Fig. 1D). This highlights that homotypic INA-6 aggregates predominantly arise through cell division."

4. Apoptosis of INA-6 Depends on Ratio Between Heterotypic and Homotypic Interaction

Original Text:

"Direct interaction with hMSCs is known to increase the survival of myeloma cells via NF- κ B signaling (15). However, little is known about how aggregation affects myeloma cell survival during hMSC-interaction. Hence, we measured cell viability (ATP) and apoptosis rates of INA-6 growing as homotypic aggregates and those growing in heterotypic interaction with hMSCs (Fig. 1E)."

Improved Text:

"While direct interaction with hMSCs is recognized to enhance myeloma cell survival through NF- κ B signaling (15), the impact of aggregation on myeloma cell survival during hMSC interaction remains less explored. Consequently, we assessed cell viability (ATP) and apoptosis rates of INA-6 cells growing as homotypic aggregates compared to those in heterotypic interaction with hMSCs (Fig. 1E)."

I'll continue with the next sections. Let me know if there are specific aspects you'd like to emphasize or if you have any preferences!



ok, you're being a great help! You can continue, I do like your input!



5. Single INA-6 Cells Detach Spontaneously from Aggregates of Critical Size

Original Text:

"Time-lapse acquisitions showed that $26\% \pm 8\%$ of observed INA-6-aggregates growing on top of single hMSCs spontaneously lost INA-6 cells (Fig. 2A, B; Vid. S1). Because all detached cells moved in a similar direction, we assumed that these cells were caught in convective streams that arose from the temperature gradients of the incubation chamber. The frequency of detachment increased after 52 h. From this time on, INA-6 cells detached mostly from other INA-6 cells (Fig. 2B), showing that homotypic interactions involved weaker adhesive forces than heterotypic interactions. Furthermore, most aggregates that lost INA-6 cells were categorized as large ($> \sim 30$ cells) (Fig. 2C)."

Improved Text:

"Through time-lapse acquisitions, we observed that $26\% \pm 8\%$ of INA-6 aggregates growing atop single hMSCs spontaneously shed INA-6 cells (Fig. 2A, B; Vid. S1). Notably, all detached cells exhibited similar directional movement, suggesting their entrainment in convective streams generated by temperature gradients within the incubation chamber. The frequency of detachment notably increased after 52 h, and from this point onward, INA-6 cells primarily detached from other INA-6 cells (Fig. 2B), underscoring weaker adhesive forces in homotypic interactions compared to heterotypic interactions. Additionally, the majority of aggregates that experienced INA-6 cell loss were categorized as large ($> \sim 30$ cells) (Fig. 2C)."

6. Cell Division Generates a Daughter Cell Detached from hMSC

Original Text:

"We suspected that INA-6 cells could detach from hMSCs immediately after cell division, since we observed that adherent INA-6 cells could generate daughter cells that "roll over" the adherent mother cell (Fig. 3A; Vid. S2). We recorded and categorized the movement of INA-6 daughter cells on confluent hMSCs after cell division and found that half of all INA-6 divisions yielded two daughter cells that did not move, indicating hMSC-adherence (Fig. 3B, C; Vid. S3). The other half of the division events generated one hMSC-adhering (MA) cell and one non-hMSC-adhering (nMA) cell that rolled around the MA cell for a median time of 2.5 h post division ($Q1=1.00$ h, $Q3=6.25$ h) until it stopped and re-adhered to the hMSC monolayer (Fig. 3D; Vid. S2, S4). Therefore, cell division creates a time window during which one daughter cell can detach."

Improved Text:

"Our observation that adherent INA-6 cells could generate daughter cells that 'roll over' the adherent mother cell prompted us to suspect that INA-6 cells might detach from hMSCs immediately after cell division (Fig. 3A; Vid. S2). We meticulously recorded and categorized the movement of INA-6 daughter cells on confluent hMSCs post-division, revealing that half of all INA-6 divisions resulted in two daughter cells that remained stationary, indicating hMSC-adherence (Fig. 3B, C; Vid. S3). Conversely, the remaining half of division events produced one hMSC-adhering (MA) cell and one non-hMSC-adhering (nMA) cell, which rolled around the MA cell for a median time of 2.5 h post-division (Q1=1.00 h, Q3=6.25 h) before re-adhering to the hMSC monolayer (Fig. 3D; Vid. S2, S4). Thus, cell division establishes a time window during which one daughter cell can detach."

I'll continue with the next sections. Let me know if there are specific aspects you'd like to emphasize or if you have any preferences!



ok perfect, please continue!



7. Well Plate Sandwich Centrifugation (WPSC) Separates hMSC-Interacting INA-6 Subpopulations

Original Text:

"To separate nMA and MA cells for further analysis, we developed the method 'Well Plate Sandwich Centrifugation' (WPSC, described in Fig. 4A). Again, we aimed to display effects of direct interaction. Hence, we seeded co-cultures in hMSC-conditioned medium (CM) from the respective donors and compared results to INA-6 incubating in CM without hMSCs. We microscopically tracked the numbers of both nMA and MA INA-6 after each separation and centrifugation step during WPSC. Both populations were sufficiently separated after the third centrifugation, whereas CM-treated INA-6 cells alone required only one centrifugation (Fig. 4B). Hence, WPSC yield cell numbers that are sufficiently high for further analysis."

Improved Text:

"To isolate nMA and MA cells for in-depth analysis, we devised the 'Well Plate Sandwich Centrifugation' (WPSC) method, outlined in Fig. 4A. Our objective was to elucidate the effects of direct interaction, prompting us to seed co-cultures in hMSC-conditioned medium (CM) from the respective donors, contrasting the results with INA-6 incubated in CM without hMSCs. Throughout WPSC, we meticulously monitored the abundance of both nMA and MA INA-6 cells following each separation and centrifugation step. The third centrifugation effectively separated both populations, while CM-treated INA-6 cells required only one centrifugation for isolation (Fig. 4B). Consequently, WPSC yields cell numbers amenable for subsequent comprehensive analysis."

8. RNAseq of non-MSC-Adhering and MSC-Adhering Subpopulations

Original Text:

"To characterize the subpopulations separated by WPSC, we performed RNAseq. We identified 1291 differentially expressed genes between nMA vs. CM, 484 between MA vs. CM, and 195 between MA vs. nMA. We validated RNAseq and found that the differential expression of 18 genes correlated with those measured with qPCR for each pairwise comparison (Fig. 4C-E, S5): nMA vs. CM [$p(16) = .803$, $p = 6.09e-5$], MA vs. CM [$p(16) = .827$, $p = 2.30e-5$], and MA vs. nMA cells [$p(16) = .746$, $p = 3.74e-4$] (Spearman's rank correlation). One of the 18 genes (MUC1) measured by qPCR showed a mean expression opposite to that obtained by RNAseq (nMA vs. CM), although the difference was insignificant (Fig. 4C)."

Improved Text:

"To delineate the characteristics of subpopulations isolated through WPSC, we conducted RNAseq, revealing 1291 differentially expressed genes between nMA vs. CM, 484 between MA vs. CM, and 195 between MA vs. nMA. Rigorous validation of our RNAseq data involved correlation with qPCR measurements for 18 selected genes in each pairwise comparison (Fig. 4C-E, S5). Robust correlations were observed for nMA vs. CM [$p(16) = .803$, $p = 6.09e-5$], MA vs. CM [$p(16) = .827$, $p = 2.30e-5$], and MA vs. nMA cells [$p(16) = .746$, $p = 3.74e-4$] using Spearman's rank correlation. An exception was noted for MUC1 in the nMA vs. CM comparison, where qPCR exhibited a mean expression opposite to RNAseq, although statistically insignificant (Fig. 4C)."



ok, using your input I have employed these revisions for section 7 to clarify the use of conditioned medium:

To separate nMA and MA cells for further analysis, we developed the method "Well Plate Sandwich Centrifugation" (WPSC), outlined in Fig. 4A. Our aim was to resolve the differences within directly MSC-interacting INA-6 subpopulations and cancel out those caused by soluble MSC-derived factors. Hence, all cultures were incubated in hMSC-conditioned medium (CM) from the respective donors and compared to INA-6 incubating in CM without hMSCs.

We microscopically tracked the numbers of both nMA and MA INA-6 after each separation and centrifugation step during WPSC. Both populations were sufficiently separated after the third centrifugation, whereas CM-treated INA-6 cells alone required only one centrifugation (Fig. 4B). Hence, WPSC yield cell numbers that are sufficiently high for further analysis.



Great refinement! This revision provides a clearer understanding of the rationale behind using conditioned medium (CM) and emphasizes the goal of resolving differences within the directly MSC-interacting INA-6 subpopulations. The inclusion of this clarification enhances the overall clarity of the methodology section. If you have any more sections or revisions you'd like assistance with, feel free to let me know!



do you have suggestions for improvement for conciseness and/or phrasing?



Certainly! Here's a revised version that aims for conciseness and clarity:

To isolate nMA and MA cells for analysis, we developed the "Well Plate Sandwich Centrifugation" (WPSC) method, detailed in Fig. 4A. Our objective was to distinguish differences within MSC-interacting INA-6 subpopulations while mitigating influences from soluble MSC-derived factors. All cultures were incubated in hMSC-conditioned medium (CM) from respective donors, allowing comparison with INA-6 cultured in CM without hMSCs.

Microscopic tracking of nMA and MA INA-6 numbers during each WPSC separation step revealed successful separation after the third centrifugation, while CM-treated INA-6 cells required only one centrifugation (Fig. 4B). Thus, WPSC generated cell numbers suitable for subsequent analysis.



OK thanks! Before we continue with the next paragraph of the results, I'd like you to look into my introduction and see if you can make it more concise?

Multiple myeloma arises from clonal expansion of malignant plasma cells in the bone marrow (BM). At diagnosis, myeloma cells have disseminated to multiple sites in the skeleton and, in some cases, to virtually any tissue (1,2). It remains unclear how myeloma cells initially disseminate. Dissemination is thought to be a multi-step process involving invasion, intravasation, intravascular arrest, extravasation, and colonization (3). To initiate dissemination, malignant plasma cells overcome adhesion, retention, and dependency on the BM microenvironment, which could involve the loss of adhesion factors, such as CD138 (4,5). BM retention is affected by multiple factors: First, chemokines (CXCL12, CXCL8) produced by mesenchymal stromal cells (MSCs) attract plasma cells and prime their cytoskeleton and integrins for adhesion (6,7). Second, solid tissues composed of extracellular matrix (ECM) proteins (fibronectin, collagens, and proteoglycans such as decorin) provide anchorage and physical boundaries to spread; however, ECM also induces diverse cell signals that can both arrest and progress the cell cycle (8–11). ECM is also prone to degradation, which is common in several osteotropic cancers, and is the cause of osteolytic bone disease. This is driven by a 'vicious cycle' that maximizes bone destruction to extract growth factors (EGF, TGF- β) stored in calcified tissue (12). Third, direct contact with MSCs physically anchors myeloma cells to the BM (3,13). Fourth, adhesion to MSCs is well known to provide essential growth and survival signals (IL-6 and ligands of B cell maturation antigen) that are required by healthy and malignant plasma cells (5,14,15). For example, the VLA4 (Myeloma)-VCAM1 (MSC)-interface activates NF- κ B in both myeloma and MSCs, inducing IL-6 expression in MSCs. For malignant plasma cells to spread to distant areas, they require, at least partially, independence from such survival signals, for example, through autocrine IL-6 signaling (16,17). In short, anchorage of myeloma to MSCs or ECM is a 'double-edged sword': adhesion counteracts dissemination, but also presents signaling cues for growth, survival, and drug resistance (18). To address this ambiguity, we developed an in vitro co-culture system that models both survival signaling and different adhesion modalities. We utilize co-cultures of primary human MSCs (hMSCs) and the myeloma cell line INA-6 because INA-6 grow as aggregates, similar to

"microtumors" proposed by Ghobrial's concept of metastasis (19), but also form tight interactions with hMSCs.

First, we characterized how hMSCs and INA6 established cell-to-cell contacts and how different growth conformations (homotypic aggregation vs. heterotypic hMSC-adherence) contribute to the survival of myeloma cells. We then introduce external forces and track INA-6 detaching from aggregates. Assuming that detached cells lack strong adhesion and represent a "disseminated" subpopulation, we developed novel techniques separating weakly and strongly adhering cells. Since this strategy resolves differences within cells in "direct" contact with MSCs caused by different growth conformations, it improves previous approaches based on transwell inserts distinguishing signaling caused by "direct" and "indirect" contact. (20).

Lastly, we characterize these subpopulations by differential gene expression, functional enrichment analysis, cell survival and validate our findings through patient survival and expression data.



Certainly! Here's a more concise version of your introduction:

Multiple myeloma results from clonal expansion of malignant plasma cells in the bone marrow (BM), yet the initial steps of dissemination are unclear. Dissemination involves a complex process encompassing invasion, intravasation, intravascular arrest, extravasation, and colonization. Myeloma cells must overcome adhesion, retention, and BM microenvironment dependency, implicating factors such as CD138 loss.

BM retention involves chemokines (CXCL12, CXCL8) from mesenchymal stromal cells (MSCs), extracellular matrix (ECM) proteins, direct MSC contact, and essential growth/survival signals. However, these anchorage mechanisms also provide cues for growth, survival, and drug resistance. To address this, we developed an in vitro co-culture system with primary human MSCs and INA-6 myeloma cells. This model explores survival signaling and adhesion modalities, considering different growth conformations.

We characterize hMSC-INA-6 cell contacts, growth conformations, and their impact on myeloma cell survival. Novel techniques separate weakly and strongly adhering cells, providing insights into the "direct" contact with MSCs. External forces induce detachment, allowing the study of a "disseminated" subpopulation. This approach improves on previous methods relying on transwell inserts, distinguishing signaling from "direct" and "indirect" contact.

Subpopulations are characterized through differential gene expression, functional enrichment analysis, and cell survival. Findings are validated using patient survival and expression data.



Thanks, but this is too concise, as key information got lost. Could you try again?



Certainly, let's find a balance between conciseness and preserving key information:

Multiple myeloma originates from the clonal expansion of malignant plasma cells in the bone marrow (BM), and the early steps of dissemination are not fully understood. Dissemination involves a multi-step process, including invasion, intravasation, intravascular arrest, extravasation, and colonization.

Critical to this process is overcoming adhesion, retention, and dependency on the BM microenvironment, potentially involving the loss of adhesion factors like CD138.

BM retention is influenced by various factors: chemokines (CXCL12, CXCL8) from mesenchymal stromal cells (MSCs), extracellular matrix (ECM) proteins, direct contact with MSCs, and essential growth and survival signals. However, these anchorage mechanisms also present challenges, providing signals for growth, survival, and drug resistance.

To address these complexities, we established an in vitro co-culture system with primary human MSCs and the myeloma cell line INA-6. This model allows us to investigate survival signaling and different adhesion modalities, considering various growth conformations.

Our study characterizes hMSC-INA-6 cell contacts, the impact of different growth conformations on myeloma cell survival, and introduces novel techniques to separate weakly and strongly adhering cells. External forces induce detachment, enabling the study of a "disseminated" subpopulation. This approach enhances previous methods, particularly those relying on transwell inserts, by distinguishing signaling from "direct" and "indirect" contact.

Subpopulations are further characterized through differential gene expression, functional enrichment analysis, and cell survival assessments. Validation is achieved using patient survival and expression data, providing a comprehensive understanding of myeloma dissemination in the BM microenvironment.



ok, let us focus on the following paragraph. I want to outline the strategy and aim of our experiments in a concise manner. Could you help me with that?

To address this ambiguity, we developed an in vitro co-culture system that models both survival signaling and different adhesion modalities. We utilize co-cultures of primary human MSCs (hMSCs) and the myeloma cell line INA-6 because INA-6 grow as aggregates, similar to "microtumors" proposed by Ghobrial's concept of metastasis (19), but also form tight interactions with hMSCs.

First, we characterized how hMSCs and INA6 established cell-to-cell contacts and how different growth conformations (homotypic aggregation vs. heterotypic hMSC-adherence) contribute to the survival of myeloma cells. We then introduce external forces and track INA-6 detaching from aggregates. Assuming that detached cells lack strong adhesion and represent a "disseminated" subpopulation, we developed novel techniques separating weakly and strongly adhering cells. Since this strategy resolves differences within cells in "direct" contact with MSCs caused by different growth conformations, it improves previous approaches based on transwell inserts distinguishing signaling caused by "direct" and "indirect" contact. (20).

Lastly, we characterize these subpopulations by differential gene expression, functional enrichment analysis, cell survival and validate our findings through patient survival and expression data.



Certainly, let's streamline the strategy and aim of your experiments:

We established an in vitro co-culture system with primary human MSCs and the INA-6 myeloma cell line to model survival signaling and diverse adhesion modalities. INA-6, forming aggregates resembling "microtumors" in Ghobrial's metastasis concept, engages in tight interactions with hMSCs.

Our approach involves characterizing hMSC-INA-6 cell-to-cell contacts under different growth conformations (homotypic aggregation vs. heterotypic hMSC adherence). External forces induce INA-6 detachment from aggregates, identifying a potential "disseminated" subpopulation lacking strong adhesion. We employ novel techniques to separate weakly and strongly adhering cells, enhancing previous methods relying on transwell inserts, which differentiate "direct" and "indirect" contact signaling.

Subsequently, we characterize these subpopulations through differential gene expression, functional enrichment analysis, and cell survival assessments. Findings are validated using patient survival and expression data, offering insights into myeloma dissemination within the BM microenvironment.



ok, using your input I've drafted this version. Any feedback?

To address this ambiguity, we developed an in vitro co-culture system that models both survival signaling and different adhesion modalities: We utilize co-cultures of primary human MSCs (hMSCs) and the myeloma cell line INA-6 because of their tight interaction, but also since INA-6 grow as aggregates, similar to "microtumors" proposed by Ghobrial's concept of metastasis (19). First, we characterized different growth conformations of hMSCs and INA6 (homotypic aggregation vs. heterotypic hMSC-adherence) and their effect on myeloma cell survival. External forces induce INA-6 detachment from aggregates, identifying a potential "disseminated" subpopulation lacking strong adhesion. We employ novel techniques to separate weakly and strongly adhering cells for subsequent analysis (differential gene expression & cell survival) and validate our findings through patient survival and expression data. To our knowledge, this is the first strategy resolving differences within cells in "direct" contact with MSCs, compared to previous methods which differentiate "direct" and "indirect" contact signaling using transwell inserts.



Your revised version is concise and effectively conveys the key elements of your experimental strategy. However, I have a suggestion to enhance clarity and flow:

"To address this ambiguity, we developed an in vitro co-culture system modeling survival signaling and diverse adhesion modalities. Our co-cultures of primary human MSCs (hMSCs) and the INA-6 myeloma cell line replicate tight interactions, and INA-6 growth in aggregates, akin to "microtumors" in Ghobrial's metastasis concept (19).

Firstly, we characterized distinct growth conformations of hMSCs and INA6 (homotypic aggregation vs. heterotypic hMSC-adherence) and their impact on myeloma cell survival. External forces induce INA-6 detachment from aggregates, revealing a potential "disseminated" subpopulation lacking strong adhesion. We employ innovative techniques to separate weakly and strongly adhering cells for subsequent analysis (differential gene expression & cell survival). Our findings are validated through patient survival and expression data. Notably, this strategy resolves differences within cells in "direct" contact with MSCs, contrasting with previous methods differentiating "direct" and "indirect" contact signaling using transwell inserts."

Feel free to use or modify it according to your preferences!



ok perfect! Now let's continue with the next section in the results



Certainly! Please provide the next section of the results, and I'll be happy to offer feedback and suggestions for improvement.



I did provide you with the results, remember? Before I introduced the introduction



I apologize for the oversight. Let's proceed with the next section of your results. Please go ahead and provide the relevant section for review.



Here's the next section:

Non-MSC-Adhering INA-6 and MSC-Adhering INA-6 Have Distinct Expression Patterns of Proliferation or Adhesion, Respectively

To functionally characterize the unique transcriptional patterns in nMA-INA6 and MA-INA6, we generated lists of genes that were differentially expressed vs. the other two subpopulations [termed nMA vs. (MA & CM) and MA vs. (nMA & CM)]. We then performed functional enrichment analysis and displayed the enriched terms as ontology clusters (Fig. 5A). nMA-INA6 upregulated genes enriched with loosely connected term clusters associated with proliferation (e.g., "positive regulation of cell cycle"). MA-INA6 upregulated genes enriched with tightly connected term clusters related to cell adhesion and the production of ECM factors (e.g. "cell-substrate adhesion"). Similar ontology terms were enriched in gene lists from pairwise comparisons (nMA vs. CM, MA vs. CM, MA vs. nMA) (Fig. 5B). In particular, nMA vs. CM (but not MA vs. CM) upregulated genes that were enriched with G1/S transition, showing that WPSC isolated nMA daughter cells after cell division.

To check for similarities between lists of differentially expressed genes from hMSC-interacting subpopulations, we performed enrichment analysis on gene lists from the overlaps (\cap) between all pairwise comparisons (Fig. 5B, S6), and showed the extent of these overlaps in circos plots (Fig. 5C). The overlap between MA vs. CM and nMA vs. CM showed neither enrichment with proliferation- nor adhesion-related terms but with apoptosis-related terms. A direct comparison of MSC-interacting subpopulations (MA vs. nMA) showed a major overlap with MA vs. CM (Fig. 5C, middle). This overlap was enriched with terms related to adhesion but not proliferation. Hence, MA-INA6 and nMA-INA6 mostly differed in adhesion gene expression.

To assess whether nMA-INA6 and MA-INA6 are regulated by separate transcription factors, we examined the enrichment of curated regulatory networks from the TRRUST database (Fig. 5B bottom). All lists were enriched for p53 regulation. Regulation by E2F1 was found only in genes

upregulated in nMA vs. CM and downregulated in MA vs. nMA. Genelists involving MA-INA6 were enriched in regulation by subunits of NF- κ B (NFKB1/p105 and RELA/p65) and factors of the immediate early response (SRF, JUN). Accordingly, NF- κ B and JUN are known to mediate cell adhesion in multiple myeloma and B-cell lymphoma, respectively (36,37). Furthermore, RELA/p65 has been reported to antagonize p53, implying a potential switch from the regulation seen in nMA-INA6 (38,39).

Taken together, MSC-interacting subpopulations show unique regulatory patterns, focusing on either proliferation or adhesion.



Thank you for providing the section. Overall, the section is well-structured and provides a detailed analysis of the transcriptional patterns in non-MSC-adhering (nMA-INA6) and MSC-adhering (MA-INA6) INA-6 subpopulations. Here are some suggestions for improvement:

1. Figures and Clarity:

- Consider providing a brief description or legend for the figures mentioned in the text (Fig. 5A, Fig. 5B, Fig. 5C, S6). This will help readers understand the visual representation of your results.
- Ensure that the figure references in the text are consistent with the actual figure numbers in your manuscript.

2. Terminology and Abbreviations:

- Clarify the abbreviation "CM" when it is first introduced in this section. While you might have defined it earlier in the manuscript, it's good practice to remind readers of the meaning, especially when it appears in different sections.

3. Sentence Structure:

- The sentence "MA-INA6 and nMA-INA6 mostly differed in adhesion gene expression" is clear, but you might consider rephrasing it for conciseness, such as "MA-INA6 and nMA-INA6 primarily differed in adhesion-related gene expression."

4. Transcription Factor Analysis:

- Provide a brief interpretation or implication of the enrichment of specific transcription factors (e.g., NF- κ B, p53) in the context of your study. This could help connect the results to the broader implications for myeloma cell behavior.

5. Logical Flow:

- Ensure that the logical flow of your findings is clear. For example, if there's a specific reason or hypothesis behind checking for similarities between gene lists from different subpopulations, briefly mention it to guide the reader through your thought process.

6. Conciseness:

- Consider condensing some sentences for conciseness while ensuring that key information is retained. This will enhance the readability of your results.

Let me know if you'd like specific examples or if you have any preferences for certain changes.



could you provide a version with improved flow and Conciseness?



Certainly! Here's a revised version with improved flow and conciseness:

Non-MS-Adhering INA-6 and MS-Adhering INA-6 Exhibit Distinct Proliferation and Adhesion Expression Patterns:

To functionally characterize the unique transcriptional patterns in nMA-INA6 and MA-INA6, we identified differentially expressed genes in pairwise comparisons [nMA vs. (MA & CM) and MA vs. (nMA & CM)]. Functional enrichment analysis revealed that nMA-INA6 upregulated genes associated with proliferation, while MA-INA6 upregulated genes linked to cell adhesion and ECM factor production. Comparisons between subpopulations (nMA vs. CM, MA vs. CM, MA vs. nMA) showed G1/S transition enrichment in nMA-INA6, indicating separation of daughter cells post-division. Overlaps between gene lists were analyzed for similarities, with major distinctions observed in adhesion gene expression between MA-INA6 and nMA-INA6.

To explore potential regulatory factors, we examined transcriptional networks from the TRRUST database. Both subpopulations exhibited enrichment for p53 regulation. Additionally, E2F1 regulation was unique to nMA-INA6, while MA-INA6 showed enrichment in NF- κ B and immediate early response factors (SRF, JUN). NF- κ B and JUN are known mediators of adhesion in multiple myeloma and B-cell lymphoma. Furthermore, RELA/p65, implicated in antagonizing p53, suggests a regulatory switch from nMA-INA6.

In summary, MS-interacting subpopulations demonstrate distinct regulatory patterns, emphasizing either proliferation or adhesion.

Feel free to let me know if you have any specific preferences or if there are particular points you'd like to retain or emphasize differently.



this is too concise, please don't omit sentences, but rather improve phrasing



Certainly! Here's a revised version with improved phrasing while maintaining the key sentences:

Distinct Proliferation and Adhesion Expression Patterns in Non-MS-Adhering and MS-Adhering INA-6:

To elucidate the transcriptional patterns unique to nMA-INA6 and MA-INA6, we identified differentially expressed genes in pairwise comparisons [nMA vs. (MA & CM) and MA vs. (nMA & CM)]. Functional enrichment analysis unveiled that nMA-INA6 upregulated genes associated with proliferation, contrasting with MA-INA6, which exhibited upregulation in genes linked to cell adhesion and ECM factor production.

Further analyses of subpopulations (nMA vs. CM, MA vs. CM, MA vs. nMA) revealed G1/S transition enrichment in nMA-INA6, suggesting the separation of daughter cells post-division. Overlaps between gene lists were scrutinized for similarities, revealing major distinctions in adhesion gene expression between MA-INA6 and nMA-INA6.

To explore potential regulatory factors, we delved into transcriptional networks from the TRRUST database. Both subpopulations exhibited enrichment for p53 regulation. Moreover, E2F1 regulation was unique to nMA-INA6, while MA-INA6 demonstrated enrichment in NF-κB and immediate early response factors (SRF, JUN). These factors, known mediators of adhesion in multiple myeloma and B-cell lymphoma, suggest a regulatory shift from nMA-INA6. RELA/p65, implicated in antagonizing p53, further hints at a distinctive regulatory landscape.

In conclusion, MSC-interacting subpopulations showcase unique regulatory patterns, highlighting distinct emphasis on either proliferation or adhesion.

Feel free to let me know if there are any specific sentences or points you'd like to modify or emphasize differently.



ok, here's the next section:

nMA-INA6 and MA-INA6 Show Increased Apoptosis Signaling Mediated by ER-Stress, p53 and Death Domain Receptors

We observed that apoptosis was increased in INA-6 growing on confluent hMSCs compared to CM-INA6 after 24 h of co-culture (Fig. 1D). Since this setup was similar to that used to separate hMSC-interacting subpopulations using WPSC, we looked for enrichment of apoptosis-related terms (Fig. 5B).

"Regulation of cellular response to stress" and "intrinsic apoptotic signaling pathway (in response to ER-stress)" are terms that were enriched in nMA vs. CM, MA vs. CM and their overlap. We also found specific stressors for either nMA-INA6 ("intrinsic apoptotic signaling pathway by p53 class mediator") or MA-INA6 ("extrinsic apoptotic signaling pathway via death domain receptor"). Therefore, apoptosis may be driven by ER stress in both nMA-INA6 and MA-INA6, but also by individual pathways, such as p53 and death domain receptors, respectively.



Here's a revised version of the section:

Increased Apoptosis Signaling in nMA-INA6 and MA-INA6 Mediated by ER-Stress, p53, and Death Domain Receptors:

Comparing INA-6 on confluent hMSCs to CM-INA6 after 24 hours of co-culture revealed heightened apoptosis (Fig. 1D), mirroring the conditions used for WPSC separation of hMSC-interacting subpopulations. Enrichment analysis for apoptosis-related terms (Fig. 5B) highlighted terms like "Regulation of cellular response to stress" and "Intrinsic apoptotic signaling pathway (in response to ER-stress)" across nMA vs. CM, MA vs. CM, and their overlap.

Specific stress-related terms such as "Intrinsic apoptotic signaling pathway by p53 class mediator" and "Extrinsic apoptotic signaling pathway via death domain receptor" were uniquely enriched in nMA-INA6 and MA-INA6, respectively. This suggests that while apoptosis in both subpopulations may be influenced by ER stress, distinct pathways, including p53 and death domain receptors, contribute individually to the process.

Let me know if there are any specific points you'd like to emphasize or modify further.

ChatGPT kann Fehler machen. Überprüfe wichtige Informationen.