Integrative Multiomics Analysis of Tumor-Immune Microenvironment in Multiple Myeloma: Novel Insights and Therapeutic Implications

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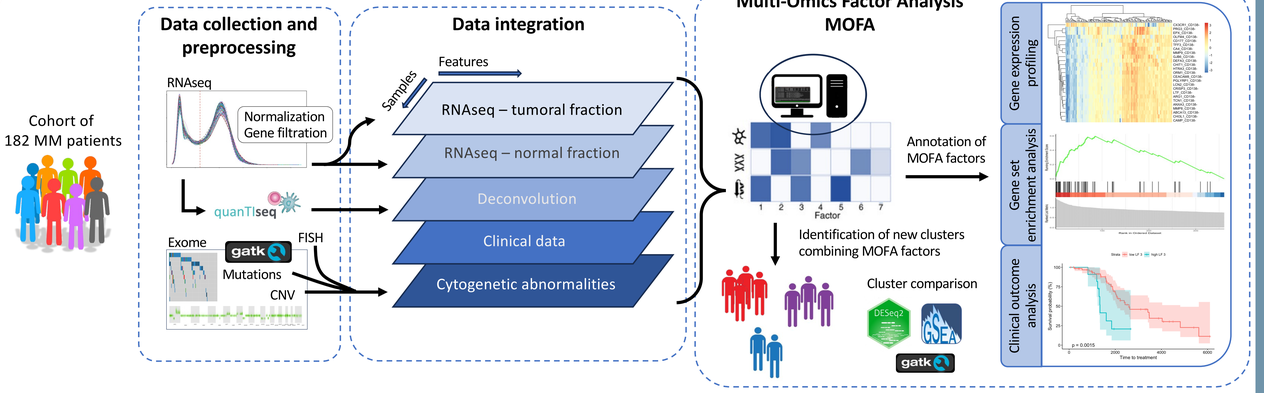
INTRODUCTION

Despite notable therapeutic advances that have improved the survival of **multiple myeloma** (MM) patients, development of drug resistance remains a major problem. **Transcriptomic analysis** provides an opportunity to dissect the complexity of tumors, including the surrounding **microenvironment**, which has a significant impact on MM tumor progression and patients' response to treatment, as demonstrated by the effectiveness of immunomodulatory therapies. To improve the tailoring of targeted and immune based therapeutic strategies, it is crucial to decrypt the **tumor-immune microenvironment profile** in MM patients.

METHOD

We used a multi-omics data integration approach, including **RNAseq-based gene expression for MM isolated-tumor cells** (MMCs) at diagnosis (n=182) and for the corresponding **isolated-tumor microenvironment** (TME) cells (n=124), **single nucleotide variant** and **copy number variation** data from **whole exome sequencing** of MMCs (n=100), **deconvolution** of TME immune subtypes (n=124), and relevant clinical metadata. This allowed us to throughly characterize both the tumor and its TME and identity new groups of MM patients



RESULTS

1. Multiomics Factor Analysis (MOFA) identified 12 principal factors capturing the diversity of the disease and highlighting a strong contribution of TME.

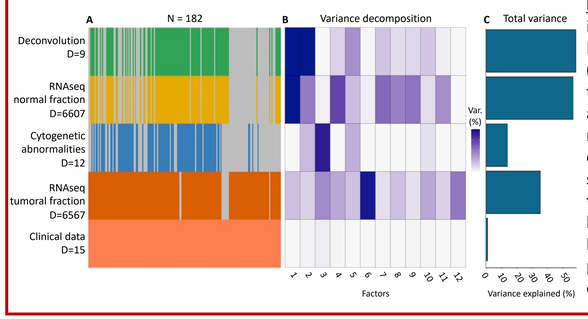


Figure 1: Multiomics data integration using a cohort of MM patients. **(A)** Study overview and data types. Each row represents an « -omics layer » with its number of features (D) and each column represents a sample. **(B)** Proportion of total variance explained (%) by individual factors for each layer and **(C)** cumulative proportion of total variance explained.

2. Association analysis with covariates demonstrated that Factor 1, which captures TME heterogeneity, is related to t(11;14) translocation and Daratumumab response, while Factors 3, 10 and 12, capturing tumor fraction heterogeneity, are related to MM progression, MM molecular subgroups and t(4;14) translocation.

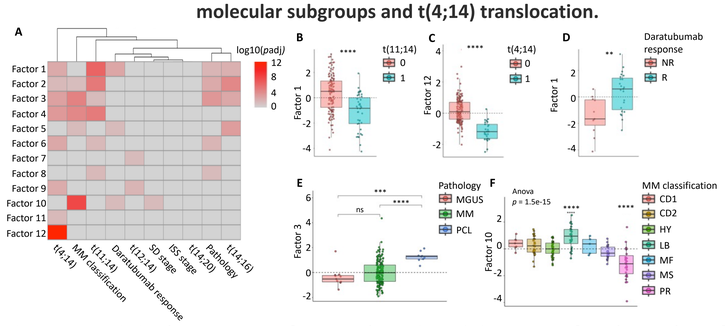


Figure 2: Association between MOFA factors and covariates. **(A)** Heatmap of p-values. Values of Factor 1 and 12 in patients with or without t(11;14) **(B)** and t(4;14) **(C)**. **(D)** Values of Factor 1 in responders and non-responders to Daratumumab. **(E)** Values of Factor 3 in monoclonal gammopathy of undetermined significance (MGUS), MM and plasma cell leukemia (PCL). **(F)** Values of Factor 10 in MM molecular subgroups according to the Zhou classification. T-test. ns: non-significant, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.0001.

3. Factor 1 showed a positive association with *MMP8* expression and genes involved in defense response by TME cells and a negative correlation with the presence of CD4 and CD8 positive T cells in the TME

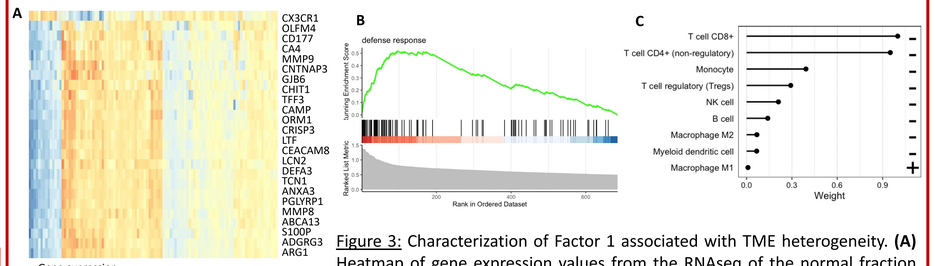


Figure 3: Characterization of Factor 1 associated with TME heterogeneity. **(A)** Heatmap of gene expression values from the RNAseq of the normal fraction for genes with the largest weights in Factor 1. **(B)** Gene set enrichment analysis of Factor 1 positive weights in the normal fraction. **(C)** Top weigths of the Factor 1 in the deconvolution layer.

4. Factor 2 exhibited positive alignments with the expression of *BHLHA15*, *TNFRSF17 (BCMA)*, *KLF15*, and *IGF1* by TME cells

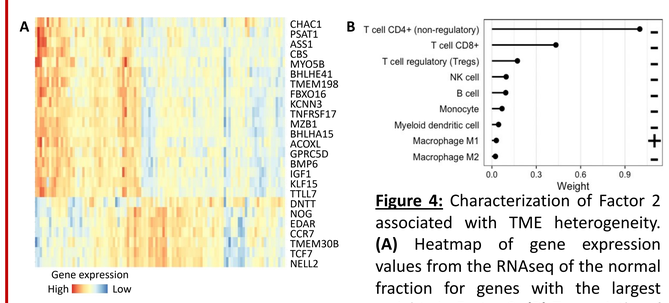


Figure 4: Characterization of Factor 2 associated with TME heterogeneity. **(A)** Heatmap of gene expression values from the RNAseq of the normal fraction for genes with the largest weights in Factor 2. **(B)** Top weigths of the Factor 2 in the deconvolution layer

5. Factor 6 capturing tumor fraction heterogeneity showed alignment with the expression of cancer testis antigens (*MAGEA6*, *MAGEA3*), *CXCL8*, and *KLF4*

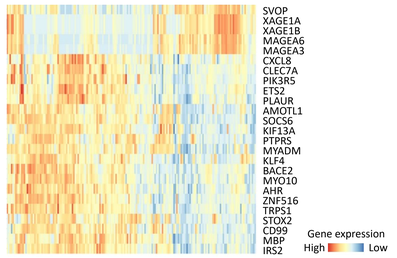
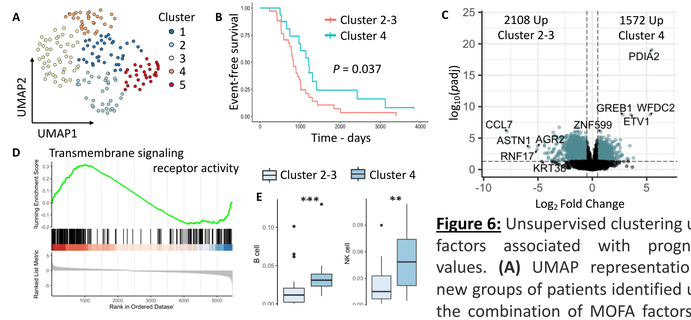


Figure 5: Heatmap of gene expression values from the RNAseq of the tumoral fraction for genes with the largest weights in Factor 6.

6. Performing unsupervised clustering using factors associated with prognostic values, we identified groups of patients with distinct TME subtypes.



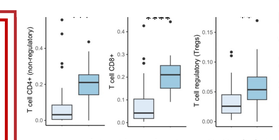


Figure 6: Unsupervised clustering using factors associated with prognostic values. **(A)** UMAP representation of new groups of patients identified using the combination of MOFA factors. **(B)** Prognostic value of the clusters 2 and 3 versus 4 (EFS, Kaplan-Meier curves). **(C)** Volcano plot of genes differentially expressed in clusters 2-3 and 4 in the normal counterpart. **(D)** Gene set enrichment analysis of DEG in cluster 4 compared to the clusters 2-3. **(E)** Immune-cell types comparison in clusters 2-3 and 4. T-test. T.test. ns: non-significant, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.0001.

CONCLUSIONS

These findings highlight the importance of TME analysis in predicting treatment response and MM patients' outcome in the context of immune-based therapies. Our integrative multi-omics analysis revealed comprehensive MM heterogeneity and distinct immune subtypes that could be of therapeutic interest for personalized therapeutic approaches.