Evidences of immune evasion in metastatic melanoma through circulating tumor miRNAs

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INTRODUCTION

In solid tumors, cancer cells are in close interaction with surrounding and infiltrating non-cancerous cells which form the tumor microenvironment (TME). The TME is capable of favourouring tumor escape mechanisms or even promoting metastasis, which greatly reduce patient's overall survival. Metastatic melanoma, for example, is a very aggressive disease with poor prognosis. Therapies based on boosting the immune antitumor response such as checkpoint blockade molecules have been successful for some patients, highlighting the importance of immune response in melanoma. Thus, it is of great interest to study the interactions between melanoma and it's TME to better understand their relationship.

METHODS

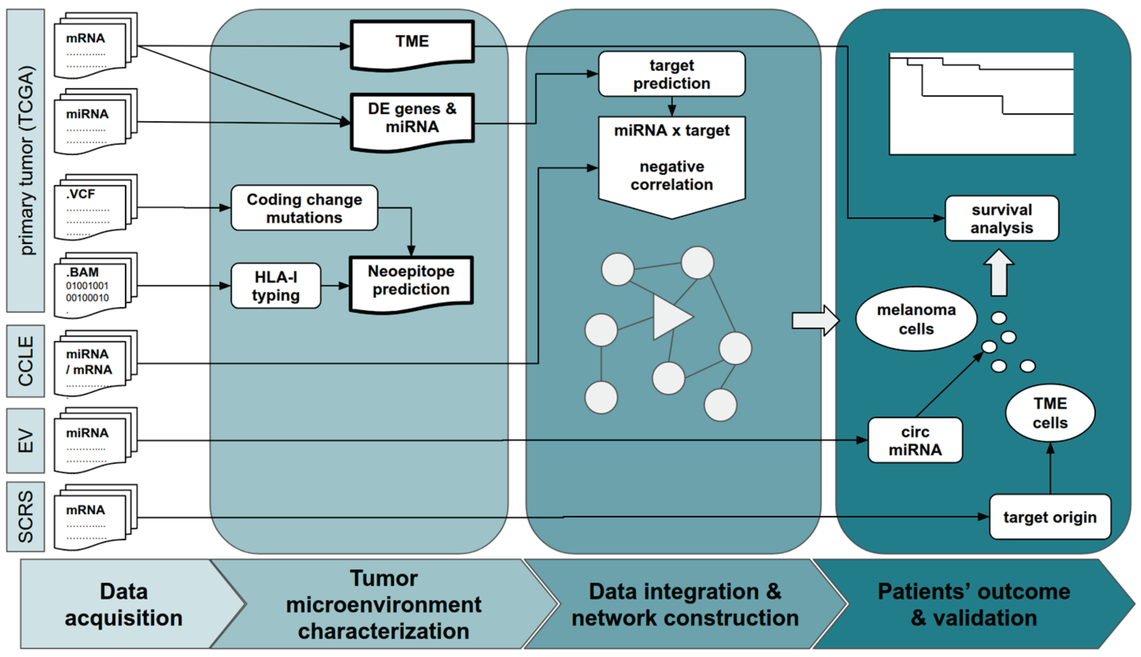

Figura 1 - Workflow summarizing the bioinformatics approach. EV - Extracellular vesicles. CCLE - Cancer Cell Line Encyclopedia. SCRS - Single-cell RNASeq. TGCA - The Cancer Genome Atlas. TME - Tumor microenvironment. DE - Differential expression. HLA - Human leukocyte antigen.

RESULTS

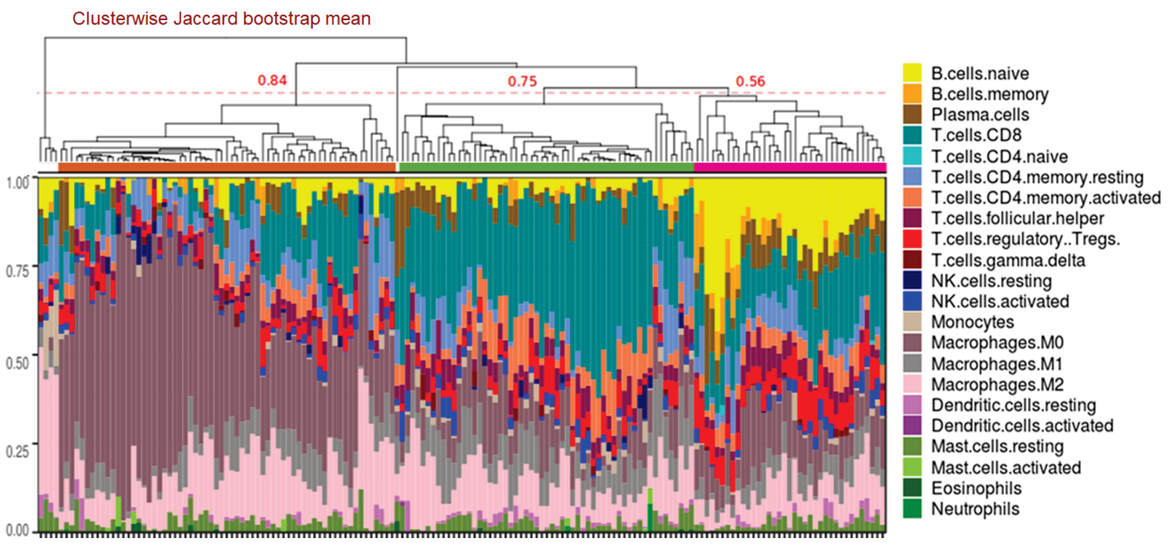

Figura 2 - Unsupervised clustering of samples based on their predicted immune cell types. The dotted red line represents the height cutoff of 0.4 for group assignment indicated in the label (G1 - pink, G2 - green, G3 - orange) or no group (white) and values represent the clusterwise Jaccard bootstrap mean values that were calculated to confirm cluster stability, with 0.75 or higher pointing to stable clusters.

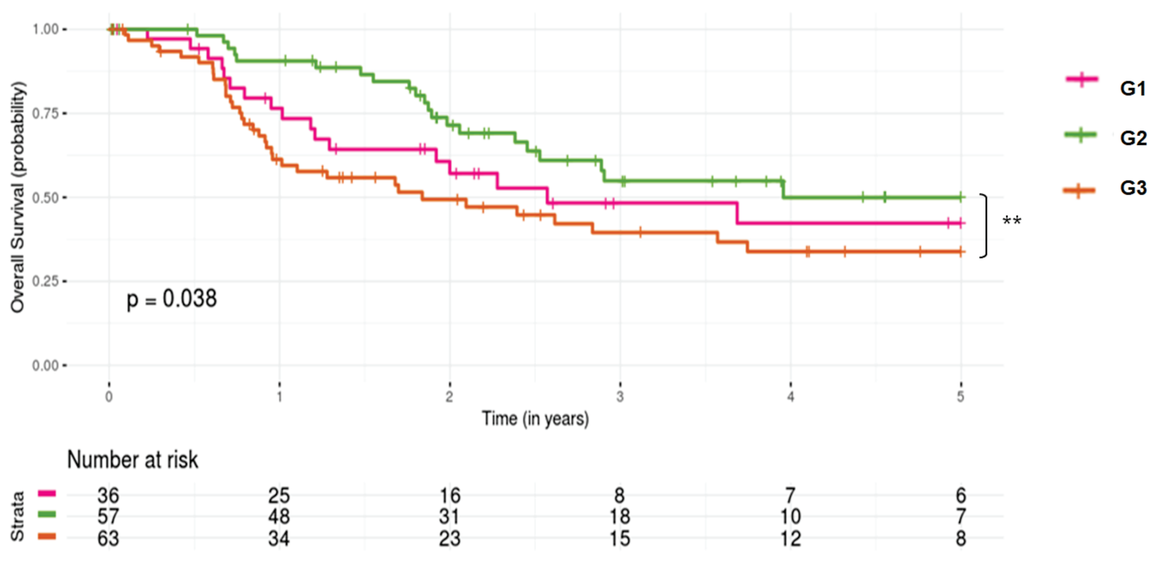

Figura 3 - Kaplan-Meier curves for 5-year overall survival rates of melanoma patients according to their TME groups. The log-rank statistic was used to test the difference in survival curves between the groups. **p-value = 0.01. Global p-value = 0.038.

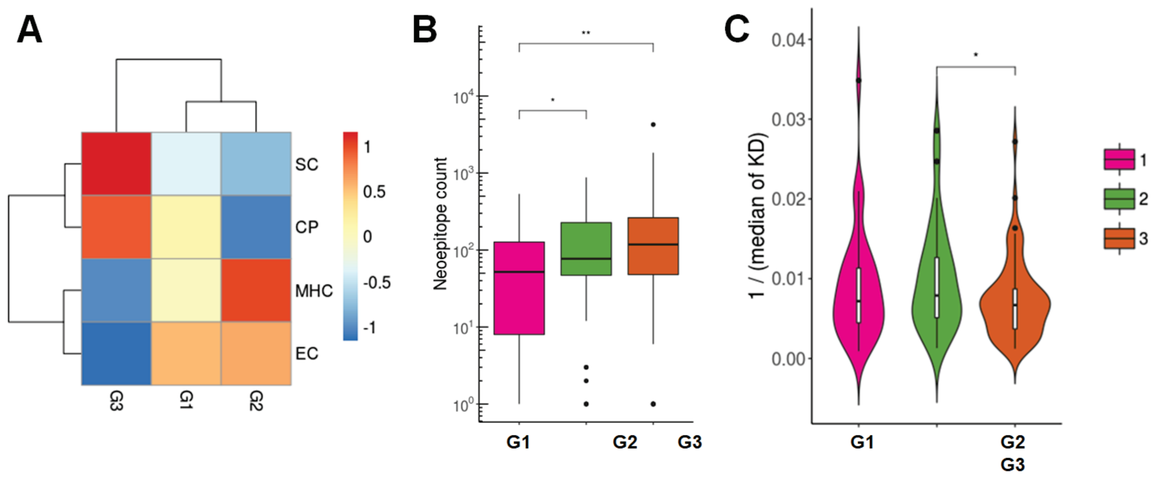

Figura 4 - Immunogenic and immune inhibitory features among groups. **a)** Immunophenoscore signature evidencing suppressor cells (SC), checkpoint molecules (CP), MHC molecules and effector cells (EC). **b)** Neoepitope burden in log scale for each group predicted by NetMHCpan through interaction of mutated peptides to HLA-I alleles. We selected the most expressed genes (upper quartile) to filter the coding change mutations generating mutated peptides prior to neoepitope prediction. The mutated peptides with a dissociation constant (KD) of less than 50nM were considered as neoepitopes. **c)** The median dissociation constant (KD) for the neoepitopes of each sample is showed for each group as 1/KD. Mann-Whitney's test was used for median comparisons. * p≤0.05, ** p≤0.01

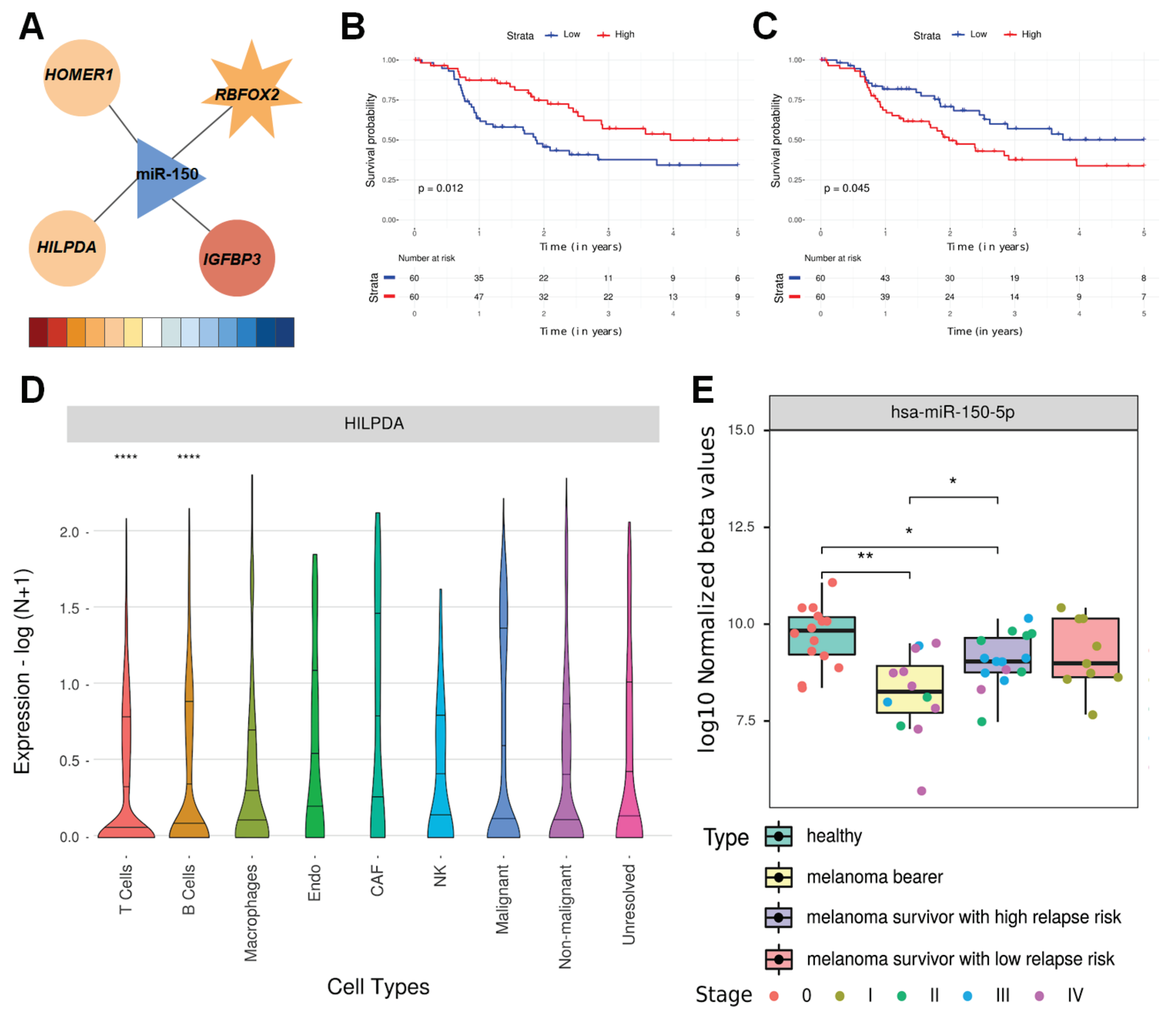

Figura 5 - Potential antitumoral role of the circulating mir-150. a) Slice of the entire miRNA/target network showing miR-150 and its predicted targets *HILPDA, HOMER1, IGFBP3* and *RBFOX2*. The circles indicate mRNAs expressed by the tumor microenvironment, the star and triangles indicate respectively miRNA-targets and miRNAs expressed by melanoma cells according to the analysis on Cancer Cell Line Encyclopedia (CCLE) database. Kaplan-Meier curves for 5-years overall survival of **b)** miR-150 and its predicted target **c)** *HILPDA* including patients from G2 and G3. Classification in groups High and Low were done taking in consideration the gene average expression. **d)** Single-cell RNA-Seq analysis of *HILPDA* expression among melanoma cells and other cell types from the tumor microenvironment. **e)** Analysis of miR-150 expression in extracellular vesicles from plasma samples comparing healthy and melanoma patients. Tumor staging is indicated (I to IV) or no tumor (0). Mann-Whitney's test was used for median comparisons. * p≤0.05, ** p≤0.01

CONCLUSIONS

In this work, we used several approaches to predict non cancerous cell populations to characterize distinguished TME profiles associated with different patients' outcome. Our results point to an immunosuppressive microenvironment in G3 characterized by the presence of low affinity neoepitopes and high expression of checkpoint genes. Moreover, through an integrative analysis, we identified miRNA-containing extracellular vesicles regulating tumor and TME cells. Among them, we found the lymphocyte-derived miR-150 potentially downregulating *HILPDA* on tumor cells, suppressing tumor growth.

Financial Support: INCA; MS, CAPES

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA