Secretome Study from BRAF^{V600E} melanoma cell lines resistant to Vemurafenib

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INTRODUCTION

Melanoma is a malignant neoplasia that is highly resistant to chemotherapy and radiotherapy and is associated with poor prognosis in advanced stage. The tumor microenvironment (TME) comprises various cell types (endothelial cells, fibroblasts, immune cells, etc.) and extra-cellular components (cytokines, growth factors, hormones, extracellular matrix, etc.) that are surrounding tumor cells and nourished by a vascular network. The TME not only plays a pivotal role during tumor initiation, progression, and metastasis but also has profound effects on therapeutic efficacy. The environment-mediated drug resistance is a result of continuous crosstalk between the tumor cells and their surrounding stroma.

In order to investigate this phenomenon in the context of acquired resistance, we obtained conditioned medium (CM) from cultured BRAF^{V600E}-mutant Vemurafenib resistant melanoma (Vem-R) cells to study if such CM contains secreted factors able to influence phenotypes and behaviours of Vemurafenib sensitive melanoma (Vem-S) cells.

MATERIAL & METHODS

Human BRAF^{V600E} melanoma cell lines sensitive to Vemurafenib treatment and Vem-R pairs were used. *In vitro* assays were performed to evaluate apoptosis, migration and invasion processes when we expose sensitive melanoma cells to the CM of the Vem-R cells. Moreover, we used plasma from SCID mice with xenografts from Vem-R melanoma cells to perform the same *in vitro* assays. A citoarray of 42 cytokines was performed to identify new possible biomarkers secreted in the media that could be involved in the resistance acquisition.

RESULTS

Secreted media from Vem-R cells conditions tumor microenvironment and induces a resistance phenotype in Vem-S cells

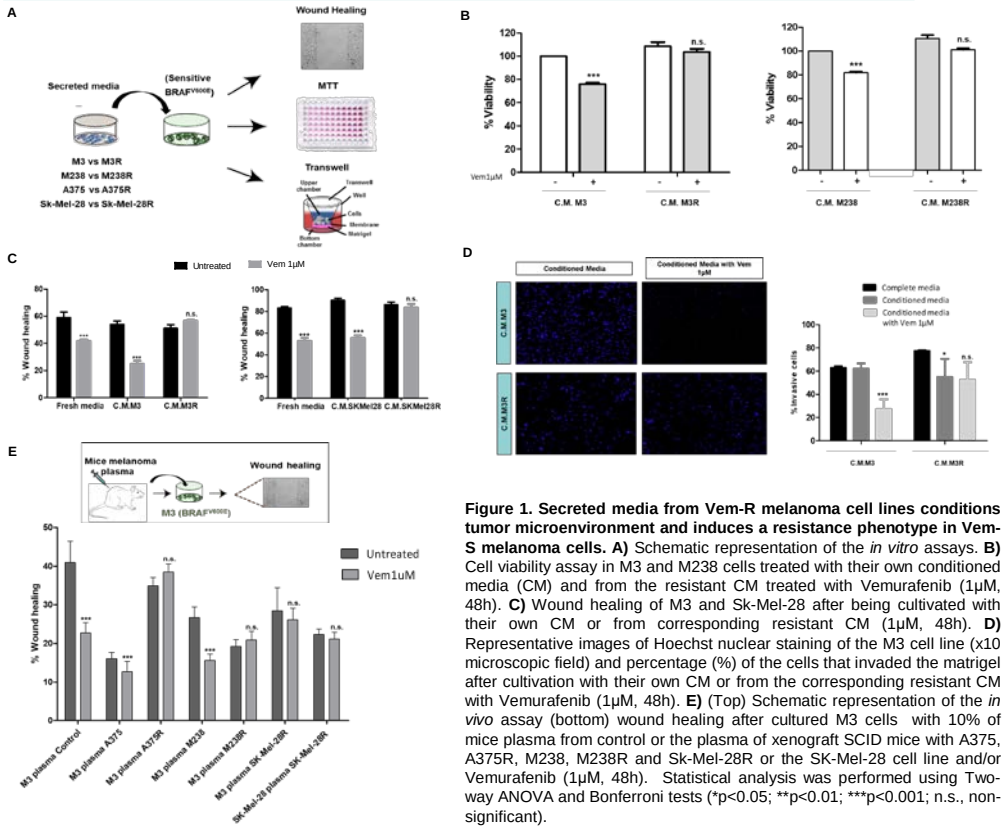

Figure 1. Secreted media from Vem-R melanoma cell lines conditions tumor microenvironment and induces a resistance phenotype in Vem-S melanoma cells. A) Schematic representation of the *in vitro* assays. **B)** Cell viability assay in M3 and M238 cells treated with their own conditioned media (CM) and from the resistant CM treated with Vemurafenib (1µM, 48h). **C)** Wound healing of M3 and Sk-Mel-28 after being cultivated with their own CM or from corresponding resistant CM (1µM, 48h). **D)** Representative images of Hoechst nuclear staining of the M3 cell line (x10 microscopic field) and percentage (%) of the cells that invaded the matrigel after cultivation with their own CM or from the corresponding resistant CM with Vemurafenib (1µM, 48h). **E)** (Top) Schematic representation of the *in vivo* assay (bottom) wound healing after cultured M3 cells with 10% of mice plasma from control or the plasma of xenograft SCID mice with A375, A375R, M238, M238R and Sk-Mel-28R or the SK-Mel-28 cell line and/or Vemurafenib (1µM, 48h). Statistical analysis was performed using Two-way ANOVA and Bonferroni tests (*p<0.05; **p<0.01; ***p<0.001; n.s., non-significant).

Cytokine secretome profile of Vem-R cells exhibits an increase in pro-tumor cytokines

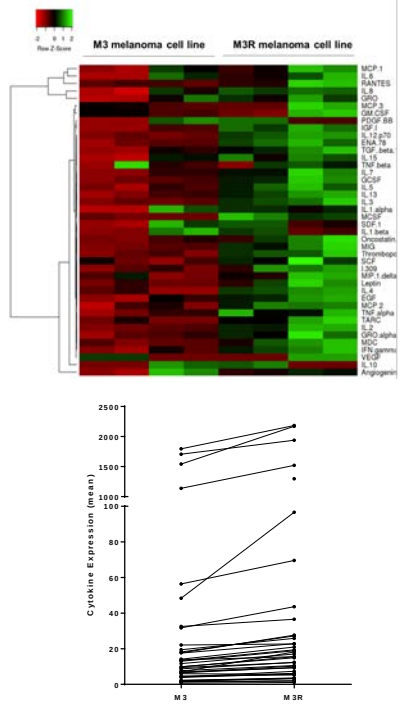

Figure 2. Cytokine secretome profile of Vemurafenib-R melanoma cell lines exhibits an increase in pro-tumor cytokines. (Top) Heatmap showing a summary of the relative levels of secretion of M3 sensitive and M3 resistant line (bottom) comparison of the cytokine secretion profile between Vem-S M3 cell line and Vem-R M3 cell line.

CONCLUSIONS

All of the data suggest that the secretion of resistant cell lines could condition the tumor microenvironment and induces the resistance phenotype in sensitive melanoma cells. Taken together, specific secreted factors from resistant cell lines could be a target therapy to overcome Vemurafenib resistance acquisition.

Acknowledgements: Supported by grants from ISCIII/FEDER "Una manera de hacer Europa" (PI1500711 to RMM & PI18/00573 to RMM & AM) and CIBERONC. PS and CB holds predoctoral fellowship from UdL-IRBLleida. IR holds a pre-doctoral fellowship from Asociación Española Contra el Cancer (AECC), Catalunya Contra el Cancer, Lleida. AM holds postdoctoral fellowship from AECC.