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A newly discovered oncomiRs miR-4443/miR-4488/nestin axis governs the migratory and invasive phenotypes of MAPKi-resistant melanoma cells

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Background

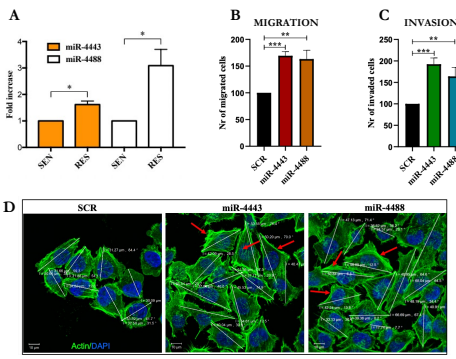
- BRAF-mutant melanoma patients benefit from the combinatorial treatments with BRAF and MEK inhibitors;
- Acquired drug resistance strongly limits the efficacy of these targeted therapies in time;
- Many findings have underscored the involvement of microRNAs as main drivers of drug resistance;
- We identified a subset of oncomiRs strongly up-regulated in drug-resistant melanomas.

Rationale

In this work, we shed light on the molecular role of two as yet poorly characterized oncomiRs, miR-4443 and miR-4488.

RESULT 2

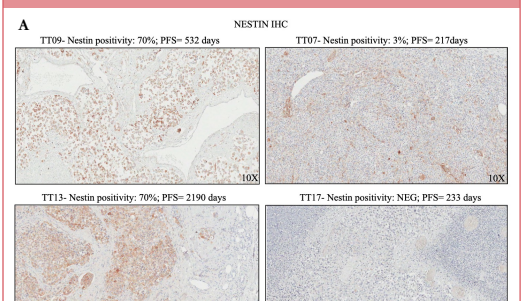
miR-4443 and miR-4488 overexpression triggers the migration and invasive potential of melanoma cells



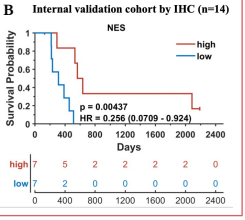
A qRT-PCR analyses of miR-4443 and miR-4488 expression levels in A375 drug-sensitive vs resistant cells confirm the upregulation of these miRNAs in A375 drug resistant cells. The values were calculated as "fold change" (± S.D.) compared to the control considered as 1. **B, C** Quantitative analyses of A375 drug-sensitive melanoma cell migration (B) and invasion (C) upon transient transfection with miR-4443 and miR-4488 by transwell assays as compared to their transfected Scrambled (SCR) counterparts. The values were calculated as "fold change" (± S.D.) compared to the control considered as 100. **D** Representative fluorescence confocal images of actin immunostaining of A375 cells transfected with miR-4443 and miR-4448. Red arrows indicate filopodia and stress fibers.

RESULT 4

Nestin high levels are predictive of a better survival for patients with BRAF-mutated melanomas

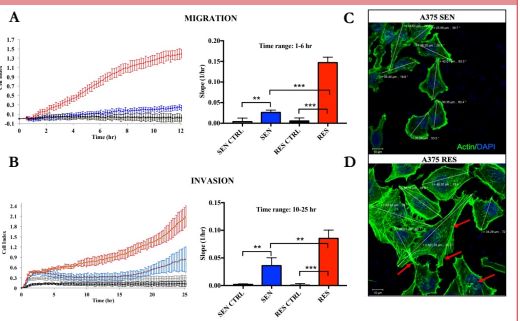


A Representative images of IHC staining for nestin levels in four melanoma biopsies coming from different patients (TT07, TT09, TT13, and TT17). 10X magnification (scale bar 500 μm). **B** Kaplan Meier curves assessing the predictive value of nestin protein levels measured by IHC in 14 melanoma patients before starting MAPKi therapy (internal cohort). All the experiments have been performed at least in triplicate and p-value <0.05 was considered as significant.



RESULT 1

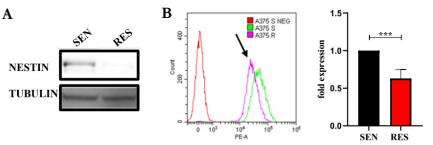
MAPKi-resistant melanoma cells show an increased migratory and invasive phenotypes as compared to sensitive ones



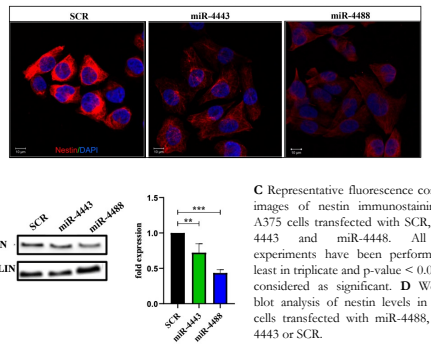
A, B Graphs (left panels) and the relative quantitative analyses (right panels) representing cell index of migrating (A) and invading (B) drug-sensitive vs resistant A375 cells obtained through xCELLigence Real Time Cell Analysis. **C, D** Representative fluorescence confocal images of actin immunostaining of A375 drug-sensitive (upper panel) vs -resistant (lower panel) cells. Resistant cells show an increased size and an increased number of stress fibers and cellular protrusions-like lamellipodia, filopodia and invadopodia as compared to sensitive cells (see red arrows).

RESULT 3

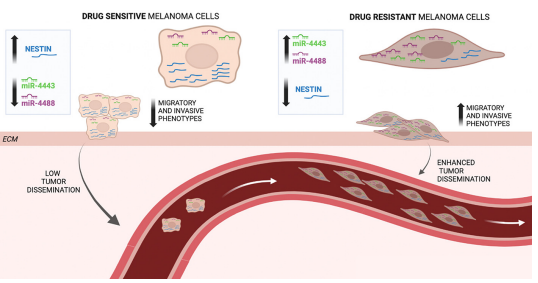
The intermediate filament nestin is reduced in drug resistant melanoma cells and is a molecular target of miR-4443 and miR-4488



A Western blot analyses of nestin levels in sensitive and resistant A375 cells. **B** Representative images of FACS analyses of nestin levels in drug sensitive vs resistant A375 cells (left panel) and the relative quantitative analyses (right panel). The values were calculated as "fold change" (± S.D.) compared to the control considered as 1.



C Representative fluorescence confocal images of nestin immunostaining of A375 cells transfected with SCR, miR-4443 and miR-4448. All the experiments have been performed at least in triplicate and p-value < 0.05 was considered as significant. **D** Western blot analysis of nestin levels in A375 cells transfected with miR-4488, miR-4443 or SCR.



Conclusions

Altogether these findings have profound translational implications in the attempt:

- to develop miRNA-targeting therapies to mitigate the metastatic phenotypes of BRAF-mutant melanomas;
- to identify novel biomarkers able to guide clinical decisions.