13

MIR-579-3P AS A CHECKPOINT FOR ADAPTATION TO TARGET THERAPY IN MELANOMA

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Background: The therapeutic landscape of BRAF-mutated metastatic melanoma has dramatically changed over the last years thanks to the advent of target therapy (MAPKi) and immunotherapies. However, the efficacy of these therapies is plagued by the onset of drug resistance. In the last years, the role of non-genetic mechanisms has emerged as adaptive events occurring early upon drug exposure. These changes drive the survival of a small fraction of slow cycling cells from which drug-resistant tumor cells emerge at later times. Among these non-genetic mechanisms, our group has demonstrated the involvement of a set of microRNAs, divided into oncomiRs and oncosuppressive miRNAs. Among the latter, our group has studied particularly miR-579-3p which is rapidly upregulated upon drug exposure and its upregulation is linked to block of cell cycle progression and inhibition of cell growth. However, its expression is lost upon prolonged drug exposure and melanoma progression. Based on these data, we hypothesize that miR-579-3p acts as a checkpoint in the process of drug adaptation.

Materials and Methods: Human melanoma A375 cell line was used to generate inducible cell line expressing miR-579-3p.
qRT-PCR and Western Blot analyses were performed to evaluate the expression of miRNA target genes. For short-term and long-term treatments, BRAFi (Dabrafenib) and MEKi (Trametinib) were used at IC_{50} concentration for A375 melanoma cells. Viability was determined by crystal violet and cell cycle analyses. For

in vivo studies, miR-579-3p encapsulated in lipid nanoparticles (LNPs) was administered systemically by i.v. injection alone or in combination with MAPKi.

Results: We showed that the induction of miR-579-3p inhibits the activation of the MAPK-ERK oncogenic pathway through the targeting of BRAF, in turn reducing cell growth in synergy with MAPKi, in short-term treatments. We also demonstrated that the constant and prolonged inducible expression of miR-579-3p in combination with targeted therapy impairs the establishment of resistance with a stable block of proliferation and cell cycle, in long-term assays. To assess the therapeutic potential of miR-579-3p *in vivo* we decided to formulate this miRNA into LNPs. This approach has been developed to overcome the rapid degradation in the bloodstream and the poor intracellular uptake of miRNAs. We demonstrated that LNP-miR-579-3p inhibits tumor growth in combination with MAPKi and significantly delays the onset of drug resistance.

Conclusion: Overall, these results suggest that miR-579-3p can be a therapeutic possibility to hit pathways responsible for drug adaptation in order to improve the efficacy of MAPKi for BRAF-mutated melanoma patients.