Circulating EV-microRNAs in Metastatic Melanoma: from diagnostic to response to treatment biomarkers

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Melanoma is the most aggressive form of skin cancer, and currently 50% of patients with metastatic or unresectable melanoma present high 5 years mortality. Thus, the identification of non-invasive and robust biomarkers of diagnosis and stratification of metastatic melanoma could be used to guide the choice of treatment. MicroRNAs (miRNAs) are small non-coding RNA molecules involved in the regulation of several cellular processes and are enriched in Extracellular Vesicles (EV). Indeed, miRNAs have been investigated in several cancers as biomarkers. This study aimed to identify EV-miRNAs as non-invasive biomarkers in metastatic melanoma and to evaluate their role in response to treatment. In this study we explored the role of EV-miRNAs as a tool to guide treatment and monitor the response in unresectable stage III and IV Melanoma patients treated with combined therapy of immune checkpoint inhibitors (ICI) and Guadecitabine (NIBIT-M4 clinical trial). Plasma was collected from treated patients before treatment (W0) and 12 weeks (W12) after treatment and EVs were isolated for microRNA profiles. Firstly, EV-miRNA profiling was performed in W0 patients' samples along with samples from a sex and age matched cohort of healthy donors. The 65 differentially expressed (DE) EV-miRNAs clearly distinguished patients from healthy controls. The enrichment analysis of the DE EV-miRNA target genes showed their involvement in migration, angiogenesis and immune regulation processes. Through a logistic regression analysis, a signature of 4 EV-miRNAs (3 up-regulated: miR-412-3p, miR-507 and miR-1203 and 1 down-regulated: miR-362-3p) capable of distinguishing metastatic melanoma patients from healthy individuals was determined. The signature was validated in an external publicly available dataset of circulating miRNAs in melanoma. A second validation was carried out on an independent internal cohort using droplet digital PCR and confirmed the differential expression of 3 miRNAs along with a good diagnostic performance of the 4 EV-miRNA signature. Next, we analyzed the EV-miRNA expression levels in patients at W0 and W12. Fifty-eight EV-miRNAs were modulated in W12 compared to W0. In addition, melanoma patients were classified in responders (R) and non-responders (NR) by clinicians and EV-miRNA profiles were compared at W12 to identify biomarkers of response to treatment. Two EV-miRNAs that were altered during treatment were associated to response to treatment of which one was of the diagnostic signature. These analyses suggest that the 2 EV-miRNAs could be useful as non-invasive biomarkers to monitor response to ICI and Guadecitabine treatment in this cohort of patients. These results highlight the role of EV-miRNAs as a non-invasive diagnostic and response to treatment tool for precision medicine. and histological analyses.