Role of miR-34a as biomarker and therapeutic agent in cutaneous melanoma

1)Carpi S. 2)Polini B. 3)Romanini A. 4)Fogli S. 5)Ylösmäki E. 6)Capasso C. 7)Breschi MC. 8)Cerullo V. 9)Nieri P.

University of Pisa

Background. MicroRNAs (miRNAs) are promising diagnostic biomarkers in cancer and other diseases and also represent therapeutic targets or drugs themselves. Their presence in blood and other physiological fluids may be a source of information useful for disease diagnosis, prognosis and treatment (Schwarzenbachet al., 2014). They are short evolutionarily-conserved regulatory RNAs whose main function is the down-regulation of proteins coded by target mRNAs (Ivkovic et al., 2017). Many miRNAs have been involved in cancer development and progression, among which miR-34a-5p (miR-34a). This miRNA is a key regulator of tumour suppression, controlling the expression of proteins involved in cell cycle, differentiation and apoptosis in different types of human cancer (Misso et al., 2014). Moreover, it down-regulates PD-L1 immune checkpoint reducing cancer immune evasion (Cortex et al., 2015). The role of miR-34a has been suggested by different authors also in cutaneous melanoma by evidences on cell proliferation and invasiveness (Liu et al., 2015). On the contrary, this miRNA has not been yet investigated as circulating biomarker and as regulator of the immune checkpoint system in this cancer.

Aim. In the present study, exosome-derived circulating miR-34a expression in plasma from metastatic melanoma patients and healthy donors was compared. Moreover, the effect of a miR-34a mimic, transfected in mouse melanoma cells and splenocytes, on PD-1, PD-L1 and Tim-3 protein expression was investigated.

Methods: After isolation of exosomes from plasma samples of melanoma patients and healthy donors, mir-34a levels were determined via quantitative real-time PCR using two types of normalization: vs endogenous miR-16 and exogenous C. elegans miR-39.

The miR-34a mimic was transfected with lipofectamine in mouse melanoma B16F10 cells and splenocytes and after 24h from transfection, PD-1, PDL-1 and Tim-3 protein expression was evaluated using a flowcytometry analysis.

Results. A statistically significant decrease in the levels of miR-34a was revealed in exosome-derived plasma of metastatic melanoma patients compared to healthy donors, observing a greater decrease in patients with BRAF V600E mutation. Preliminary in vitro experiments on cells transfected with the miR-34a mimic, revealed a reduced expression of the immune checkpoint proteins.

Conclusions. MiR-34a has a role of circulating biomarker in plasma of cutaneous melanoma patients and may represent a therapeutic agent reducing not only the proliferation of cancer cells but also their immune system escape. Future experiments will be aimed at investigating this therapeutic strategy in an in vivo melanoma model.

Keywords: miR-34a, cutaneous melanoma, exosomes, PD-1, PDL-1, Tim-3

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