miR-214 as a driver of hyperpigmentation and therapy resistance in cutaneous melanoma

Elena Andreucci[1], M. Lulli[1], Alessio Biagioni[1], Lido Calorini[1], Elisabetta Gambale[2], Serena Pillozzi[2], Lorenzo Antonuzzo[2].

[1]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy;
[2]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

INTRODUCTION

Melanin affects the clinical course of cutaneous melanoma, being hyperpigmentation related to drug resistance. Indeed, the products of melanogenesis have been known to have immunosuppressive effects, which can affect response to immunotherapy and melanoma cells undergoing drug resistance show hyperpigmentation. Several efforts have been made to discover the melano-miRs involved in melanoma progression, but they are still to be defined. miR-214 contributes to melanoma initiation and coordinates signalling critical for treatment resistance. This study aims to verify whether miR-214 promotes hyperpigmented/ resistant melanoma phenotype and to evaluate miR-214 levels in the plasma of patients with differentially pigmented lesions.

METHODS

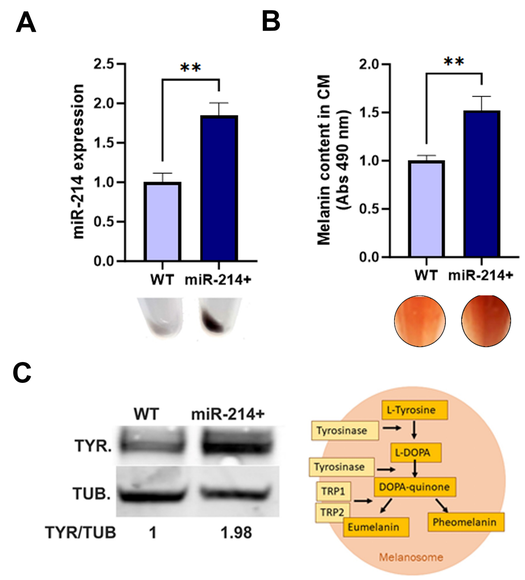
Murine B16-F10, and human 501Mel and Sk-Mel-28 melanoma cells transfected with pCMV-miR-214 plasmid were sorted for GFP and validated in qPCR for increased miR-214 expression. Cellular melanin content was evaluated in spectrophotometry, iron levels were evaluated by colourimetric assay, and ROS production was detected in flow cytometry. TargetScanHuman software suggested putative miR-214 targets involved in melanoma hyperpigmentation which were validated in western blot. Therapy response was determined by MTT, colony formation, and live/dead assays. miRNA isolation kit and subsequent qPCR were used to isolate and quantify the miR-214 expression levels in the plasma of melanoma patients.

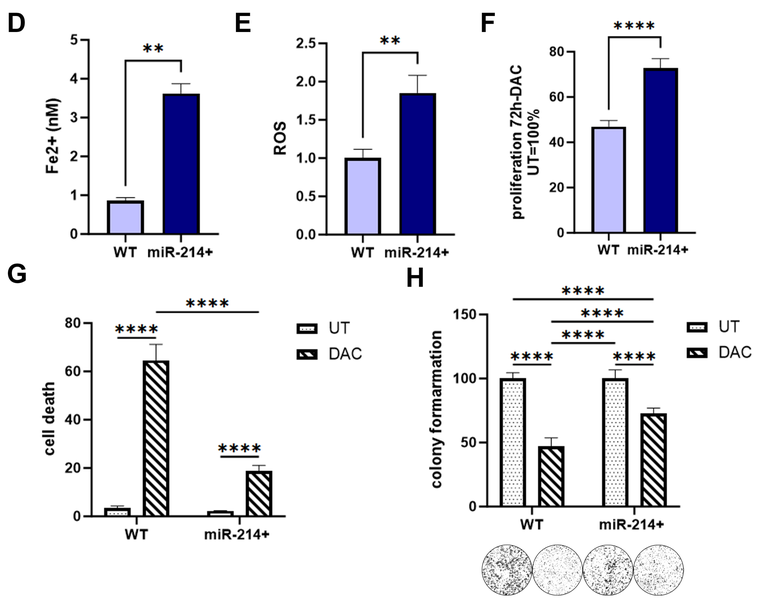
CONCLUSION

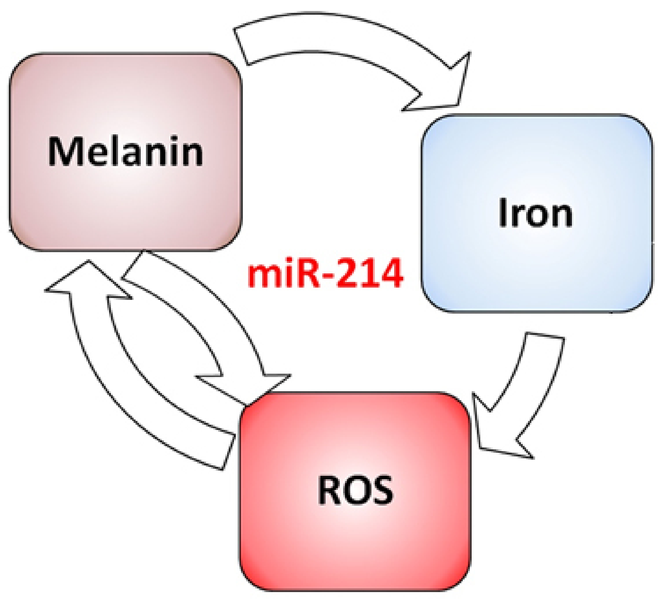
Preliminary evidence suggests that miR-214 induces hyperpigmentation that could represent an easily accessible biomarker of drug resistance

RESULTS

Melanoma cells transfected with miR-214 over-epression plasmid were validated for the increased miR-214 expression by qPCR analysis. We observed that miR-214-over-expressing cells (miR-214+) are characterized by hyperpigmentation (A-C). miR-214+ cells also show an increased content of Fe2+(D) that can account for the augmented ROS production observed (E), possibly related to both hyperpigmentation and drug resistance (F-H). Among miR-214 putative targets, we found Glutathione S-transferase Zeta 1 (GSTZ1) (i), involved in the anti-oxidant response and also the phenylalanine/ tyrosine catabolism (i.e., the main melanin precursors). Our preliminary results confirm a low level of miR-214 in the plasma of patient with non-pigmented MC compared to three patients with pigmented lesions (J).







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Dr. Elena Andreucci, PhD
Department of Experimental and Clinical Biomedical Sciences "Mario Serio",
Viale GB Morgagni, 50, 50134 Florence, ITALY
phone: +39 055 2751310
e-mail: e.andreucci@unifi.it

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