MiR-30a is an aged-related microRNA that impairs differentiation and induces apoptosis in human epidermis.

C. Muther [1], M. Garion [1], L. Jobeili [2], O. Damour [2], T. Nedachi [3], J. Lamartine [1]

[1]Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique, UMR 5305, CNRS – University of Lyon, Lyon, France
[2]Laboratoires des substituts cutanés, Hospices Civils de Lyon, Hôpital Edouard Herriot, Lyon, France
[3]Toyo University, Tokyo, Japan

muther.charlotte@gmail.com ; jerome.lamartine@ibcp.fr

Introduction

The age-related alterations of epidermis are essentially characterized by a defect in the tissue renewal caused by a reduced keratinocytes proliferation and by a disturbance of their differentiation. However, the mechanisms disturbing epidermal homeostasis during aging remain poorly understood. To go further into this question, we focused on microRNAs (miRNAs), a class of non-coding RNAs known to play a key role in the regulation of epidermal homeostasis.

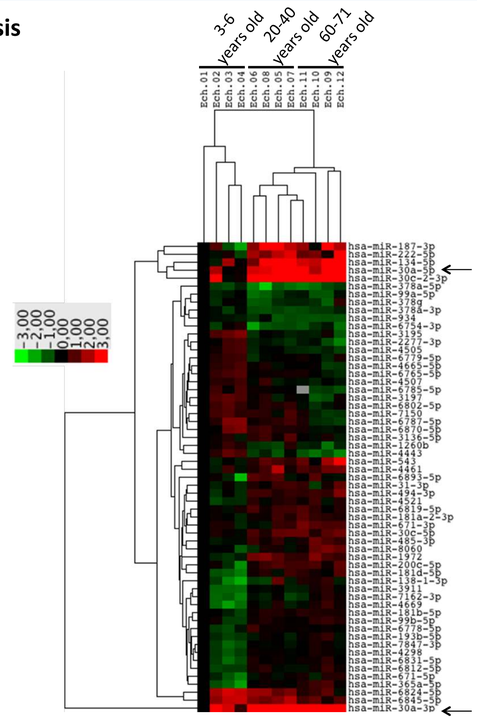
Materials & Methods

To identify age-related microRNAs, we performed an exhaustive miRNAs microarray expression screen in human primary keratinocytes from young or elderly peoples. Then, we constructed a lentiviral vector allowing inductible and stable overexpression of one microRNA which is overexpressed in aged keratinocytes, aged epidermis and in a reconstructed skin model mimicking chronological aging. Transduced keratinocytes allowed us to perform many functional tests to characterise the effects of this microRNA during epidermal aging. Putative targets of miR-30a were selected from transcriptomic data deposited in GEO database and validated in human keratinocytes.

Results

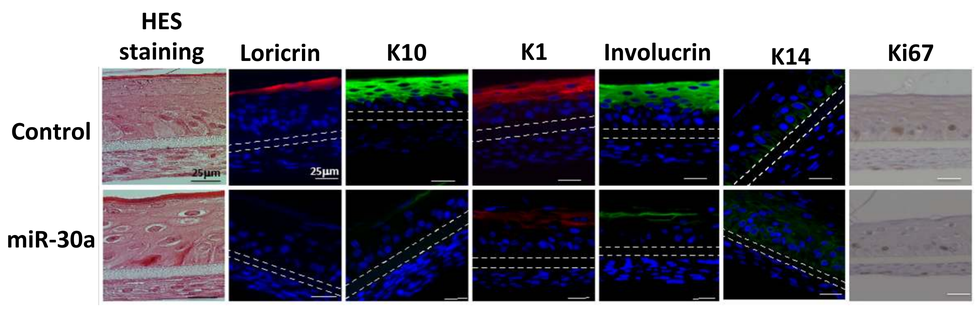
Several microRNAs, including the two strands of miR-30a, are differentially expressed in aged keratinocytes.

Microarray analysis



A miRNA microarray screening identified 60 miRNAs including miR-30a strands (arrows) significantly modulated ($p<0.05$, fold change > 1.5) between young and adult donors or between young and aged donors.

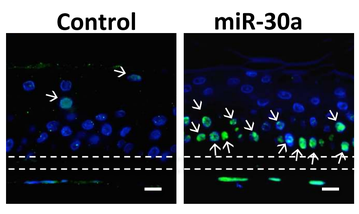
MiR-30a overexpression impairs epidermal differentiation

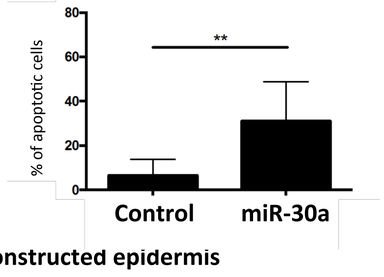


Transduced keratinocytes with a control lentivirus or a miR-30a lentivirus, allowing its stable overexpression, were used to produce reconstructed epidermis. MiR-30a overexpression induces an abnormal epidermis with parakeratosis, big round cells in suprabasal layers with absence of granular layer and impairs strongly the differentiation process.

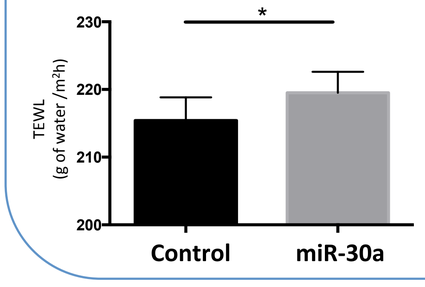
MiR-30a regulates keratinocytes apoptosis and barrier function efficiency in reconstructed epidermis

Tunel assay in reconstructed epidermis





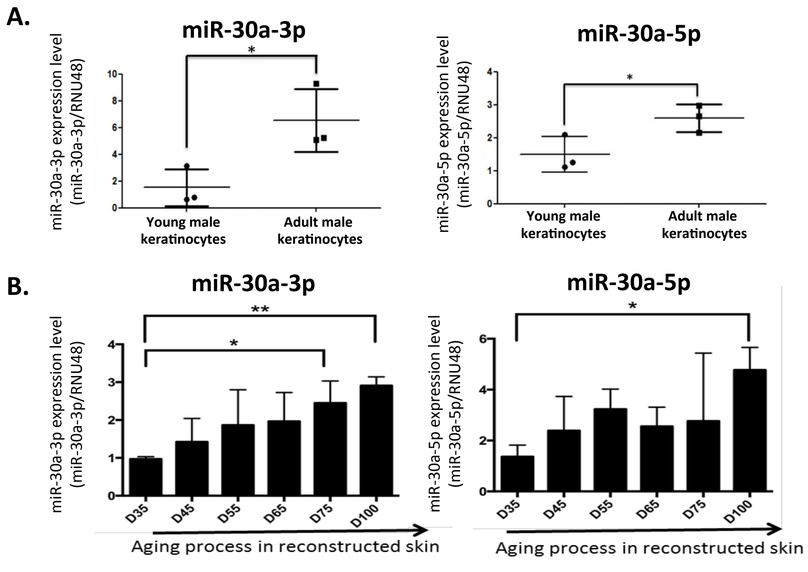
Trans-epidermal water loss (TEWL) of reconstructed epidermis



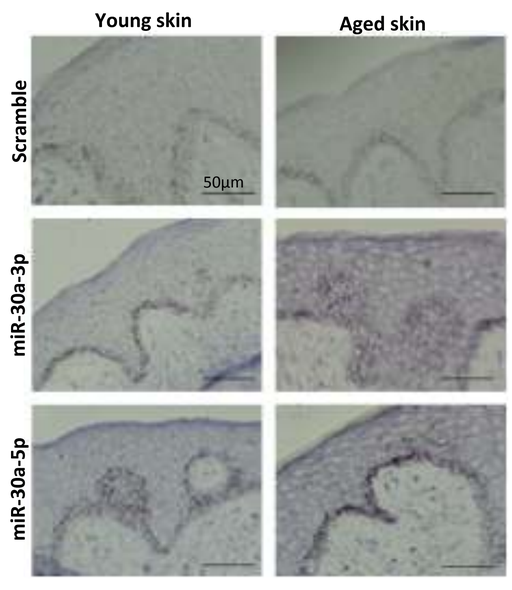
MiR-30a overexpression increases apoptotic cell abundance and impairs barrier function efficiency in reconstructed epidermis.

MiR-30a is overexpressed in aged keratinocytes (A), in a reconstructed skin model mimicking aging (B) and in aged epidermis (C)

Real-time quantitative PCR in keratinocytes and in reconstructed skin mimicking aging



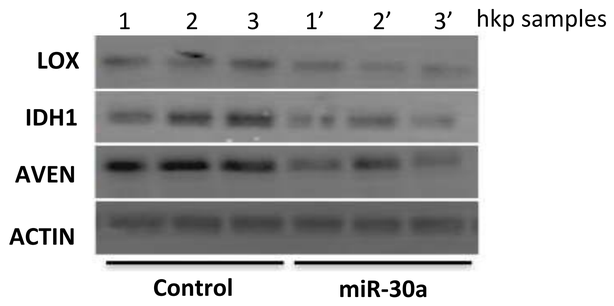
C. *In situ* hybridization in human skin



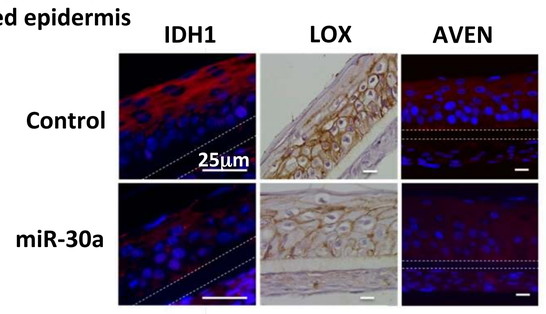
The two strands of miR-30a are overexpressed in aged keratinocytes (A) and progressively but significantly increased during culture of reconstructed skin model mimicking aging[1] (B).
In situ hybridization of miR-30a-3p and miR-30a-5p in young and aged skin showed a clear induction of the two strands of miR-30a in aged skin (C).

MiR-30a gene targets AVEN, IDH1 and LOX in keratinocytes and in reconstructed epidermis

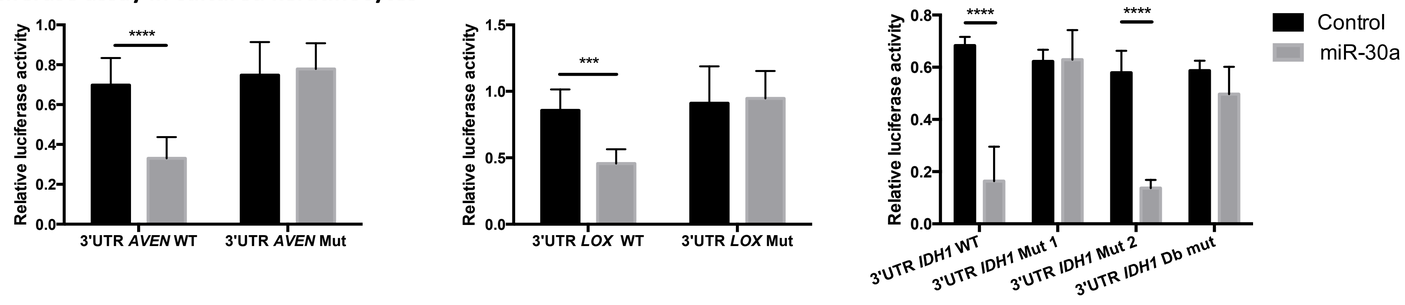
Western blot of cultured keratinocytes



miR-30a targets expression is decreased in miR-30a overexpressing reconstructed epidermis



Luciferase assay in cultured keratinocytes



AVEN (a caspase inhibitor), *LOX* (which plays a role in epidermal differentiation) and *IDH1* (an enzyme of cellular human metabolism) are direct targets of miR-30a in epidermis.

Discussion

Our work revealed a new miRNA actor and deciphered new molecular mechanisms to explain some alterations observed in epidermis during aging, especially those concerning keratinocytes differentiation and apoptotic death.
Our next objective will be to develop a new tool to turn off miR-30a expression, using CRISPR/Cas9 technology to study the potential rejuvenating effect of this miRNA.

Reference and funding

1. Dos santos and al., Matrix Biol. (2015)

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