

Biomechanical properties improved through dermal microRNA control

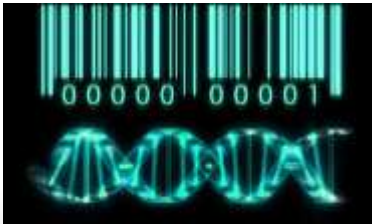
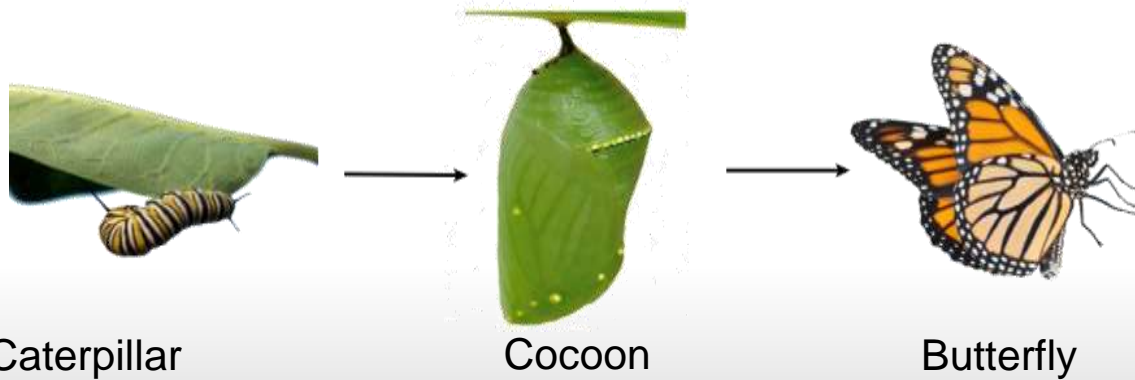
ADEBIOTECH - Romainville | March 13, 2018
Reymermier Corinne - Gaelle Saint-Auret

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 **BASF**
We create chemistry

Epigenetics

Stably heritable phenotype resulting from changes in a chromosome
without alterations in the DNA *sequence*

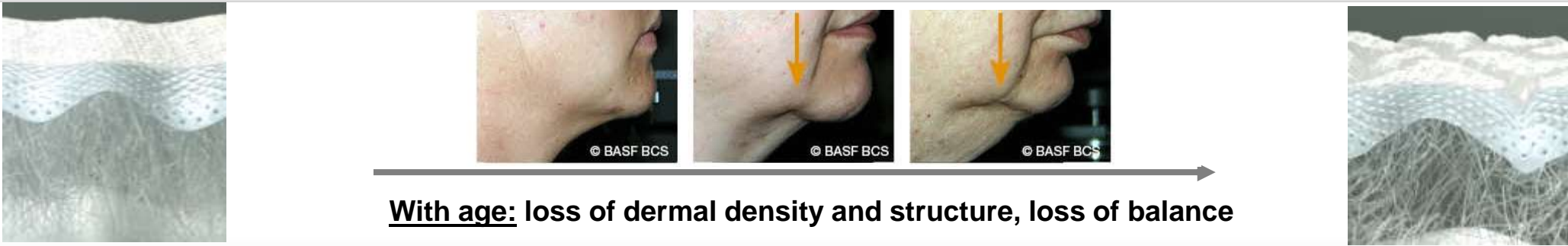


DNA:
Set of codes



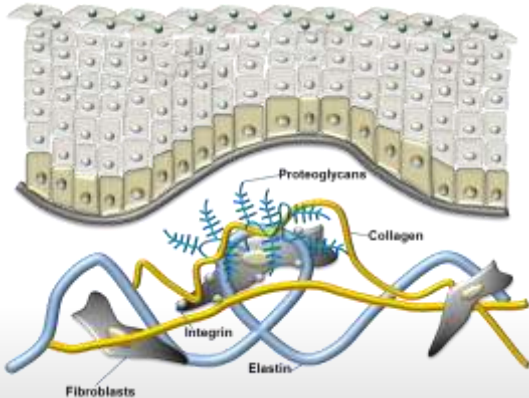
Epigenetics:
Chemical marks that dictate which
codes can be read

Age brings deregulation of protein production & skin sagging



With age: loss of dermal density and structure, loss of balance

➔ **Visible signs: SKIN SAGGING, loss of biomechanical properties**



Key elements in the dermis structure

- ➔ **Fibers (collagen & elastin)**
- ➔ **Inter-fiber cement (GAG)**
- ➔ **Cell-fiber connectors (integrin)**

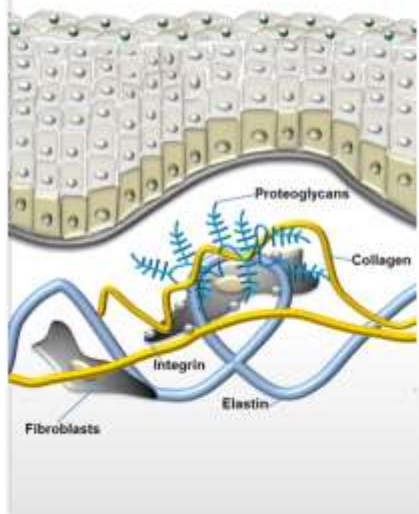
➔ **ALL AFFECTED BY AGE**

Which miRNAs should be modulated to find back skin properties?

Let-7b is the dermal regulator for skin architecture improvement

Bio-informatic selection

- ✓ Of proteins whose mRNA have **sequence homology** with **Let-7b** miRNA sequence
- ✓ Of **proteins** favors for fibers organization



Protein Code	Name	Interacting partners	Biological functions
CHSY3	Chondroitin sulfate synthase 3	Collagen and elastic fibers	Sulfatation of chondroitin sulfate
XYLT1	Xylosyl transferase-1	Collagen and elastic fibers	GAG chain assembly
TGFBR2	TGFbeta2 receptor	Collagen fibers	Wound healing and tissue repair
FBN1	Fibrillin1	Elastic fibers and integrin	Skin elasticity
ITGB3	Integrin beta3	FBN1 and cell matrix	Cell shape and ECM deposition

- The two proteins **CHSY3** and **XYLT1** are responsible for chondroitin sulfate synthesis and sulfatation necessary for GAG chains assembly in proteoglycans such as decorin or biglycan involved in **collagen and elastic fiber organization**
- **TGFBR2** knock-down results in a slowdown of wound contraction, reduced and delayed **dermal collagen organization**.
- **FBN1** is a major glycoprotein component of microfibrils that forms a template for tropoelastin during **elastic fibrillogenesis** to ensure **skin elasticity**.
- **ITGB3** participates in the integrin complex and is involved in **cell matrix interactions** through FBN1 binding, influencing **cell shape and ECM deposition**

Let-7b miRNA regulation could improve

▪ **FIBERS**

Formation of collagen fiber

- ✓ **Collagen 1**
- ✓ Protein **TGFBR2**: favors collagen organization

Formation of elastic fiber

- ✓ Fibrillin 1 (protein **FBN1**): provides a scaffold for deposition of elastin

▪ **INTER-FIBER CEMENT**

Inter-fiber cement – GAG (proteoglycan) that bring suppleness to the dermis network.

The GAGs are built by "builder proteins":

- ✓ **CHSY3**
- ✓ **XYLT1**

▪ **CELL-FIBER CONNECTOR**

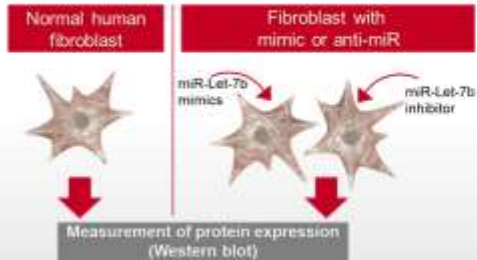
- ✓ **ITGB3**: integrin protein: Creates the links between cells and fibers for a strong dermis network.

Let-7b, a microRNA increased with aging and fibroblast quiescence (Nishino et al., 2008; Suh et al. 2012) **could be a relevant dermal regulator for skin architecture improvement**

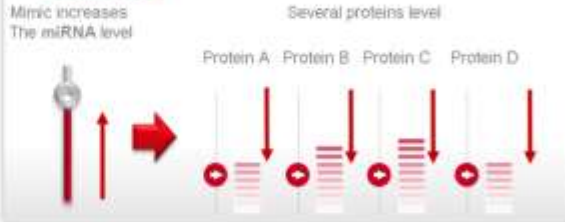
Proteins modulation for miRNA functional validation

Concept proof: Modulation of the protein synthesis

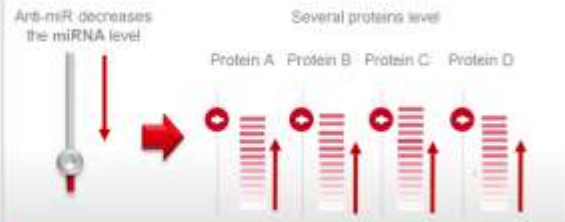
- To prove that a protein synthesis is controlled by a miRNA in a cell (epigenetic regulation), we introduce a "miR-mimic" or "miR-inhibitor" to artificially increase or decrease the production of this protein.



- Functional regulation of mRNA by the mimic miRNA is validated when the protein level decreases



- Functional regulation of mRNA by the anti-miR is validated when the protein level increases



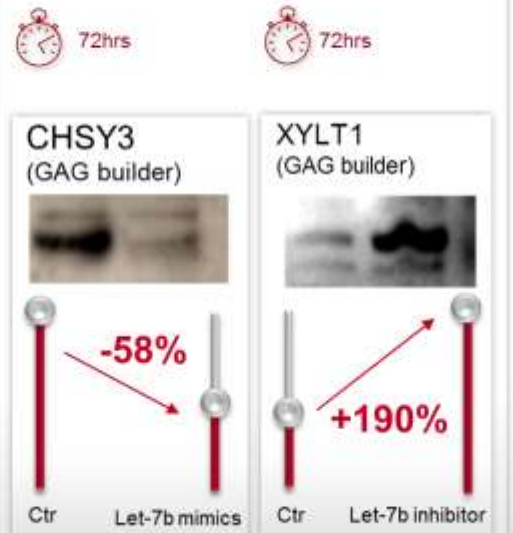
Proteins modulation = functional validation of mRNA and miRNA interactions

The Let-7b miRNA is involved in the balance of dermal architecture

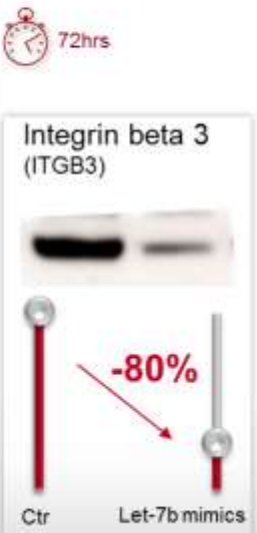
FIBERS



INTER-FIBER CEMENT



CELL-FIBER CONNECTOR

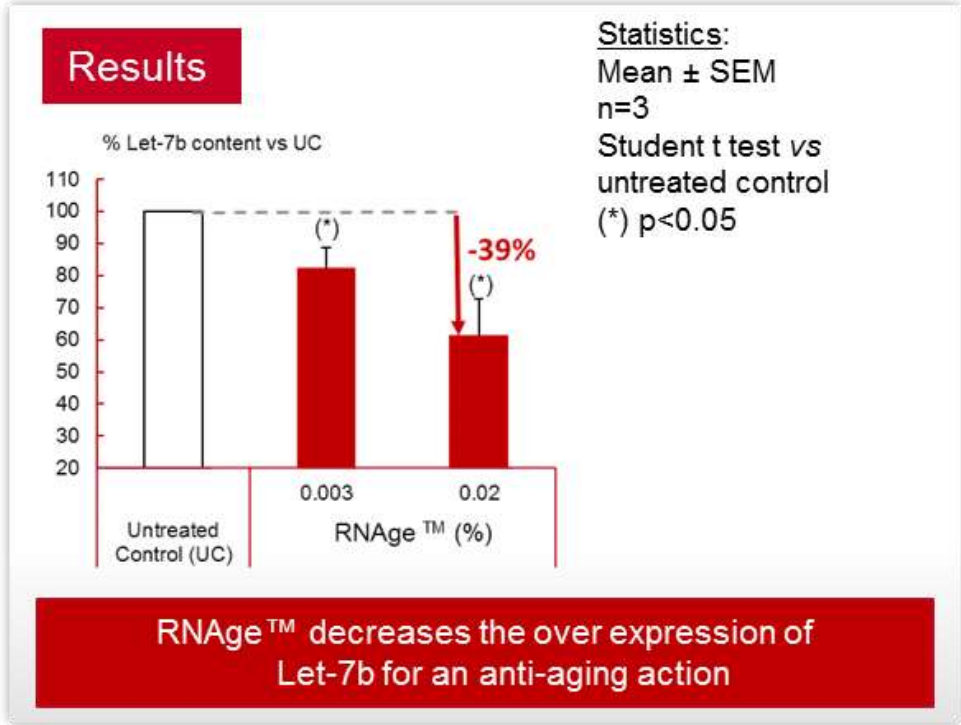
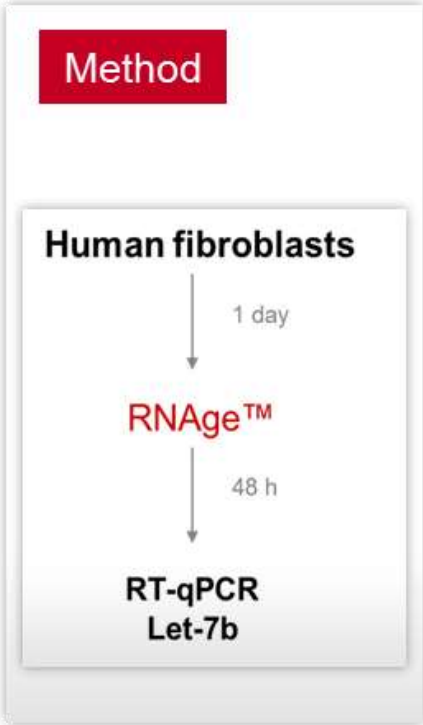
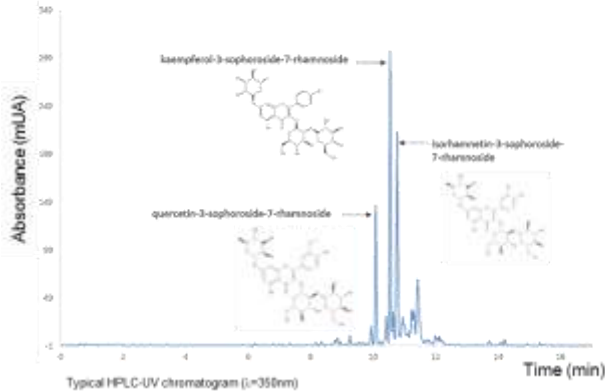


We evidenced that Let-7b controls 5 key proteins involved in the dermal architecture

The epigenetic regulation for a skin rejuvenation



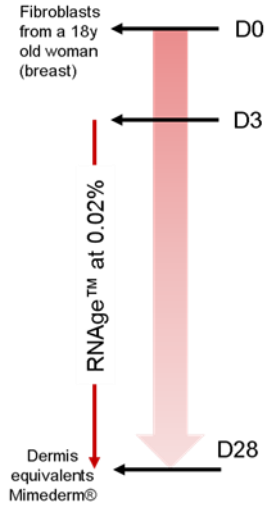
Flavonol glycosides phytochemical profile



RNAge™ is able to decrease the expression of the epigenetic skin regulator Let-7b in order to re-induce the production of proteins involved in 3D dermal architecture

Fiber cohesion, dermal density & biomechanical properties

Method

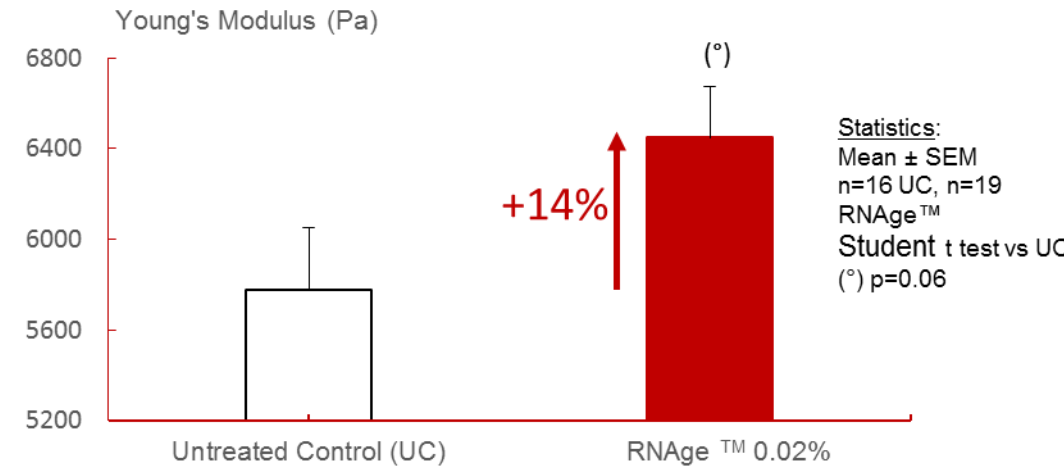
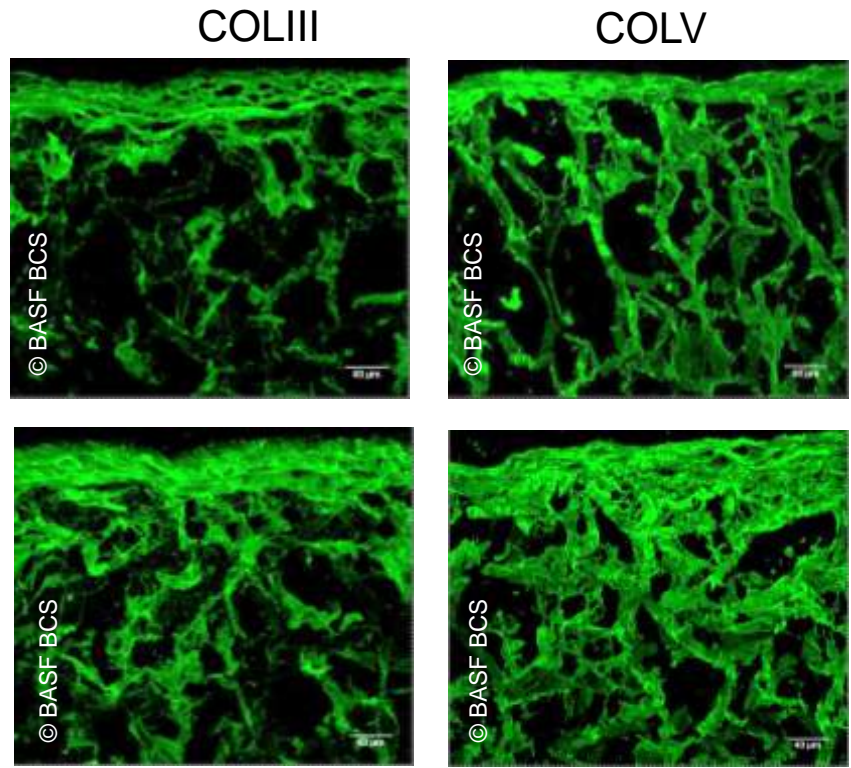


COL III & COL V confocal images & Compression test

Results

Untreated control

RNAge™



RNAge™ at 0.02% showed an improvement of 14% of the Young's modulus vs untreated control: **increased density and structure**

RNAge™ is able to increase the dermal density through the fiber cohesion involved in 3D dermal architecture and increases the biomechanical properties of the reconstructed dermis


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