MicroRNAs: novel regulators in skin research

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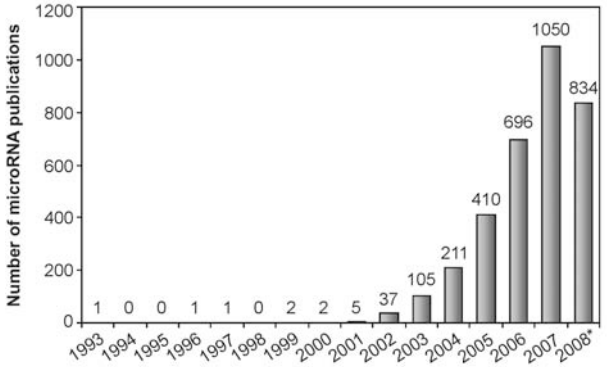
What are microRNAs?

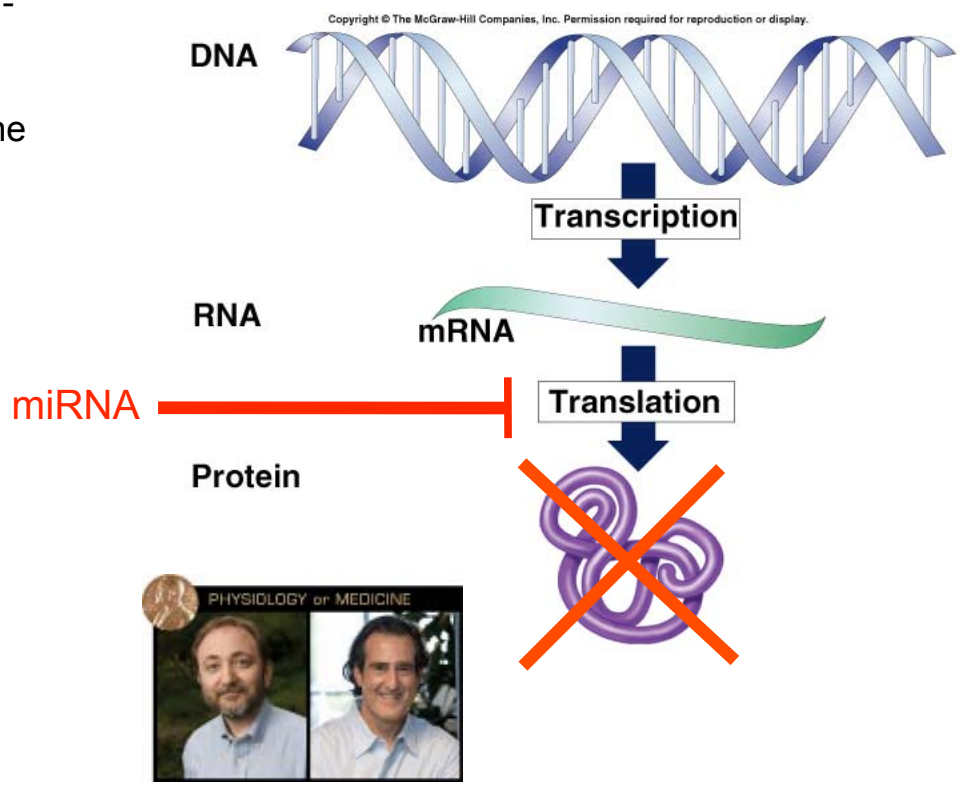
- Small, ~21-mer RNAs
- 1993: The first miRNA discovered, Lin-4, regulated the developmental transitions in *Caenorhabditis elegans*
- The second microRNA was discovered 7 years later (2000)
- Today (October, 2008) we know more than **8000 miRNAs** (miRBase 12.0)
- The most abundant regulators of gene expression in the genome!



Lee et al., *Cell*, 1993
Sonkoly et al, *J Cell Mol Med*, in press

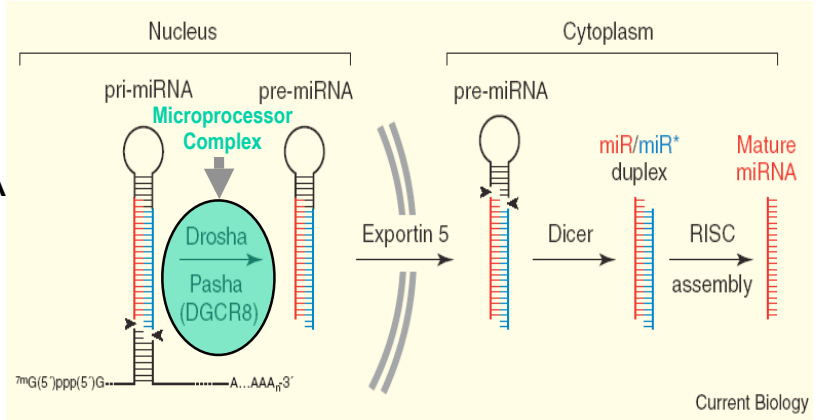
MicroRNAs: regulators of gene expression

- Regulate the expression of most protein-coding genes
- MicroRNAs inhibit gene expression at the post-transcriptional level
- Their function and importance can be compared to transcription factors
- Regulate basic biological processes
  - Apoptosis
  - Morphogenesis
  - Proliferation
  - Metabolism
  - Signal transduction
  - Determination of cell fate
  - Developmental timing



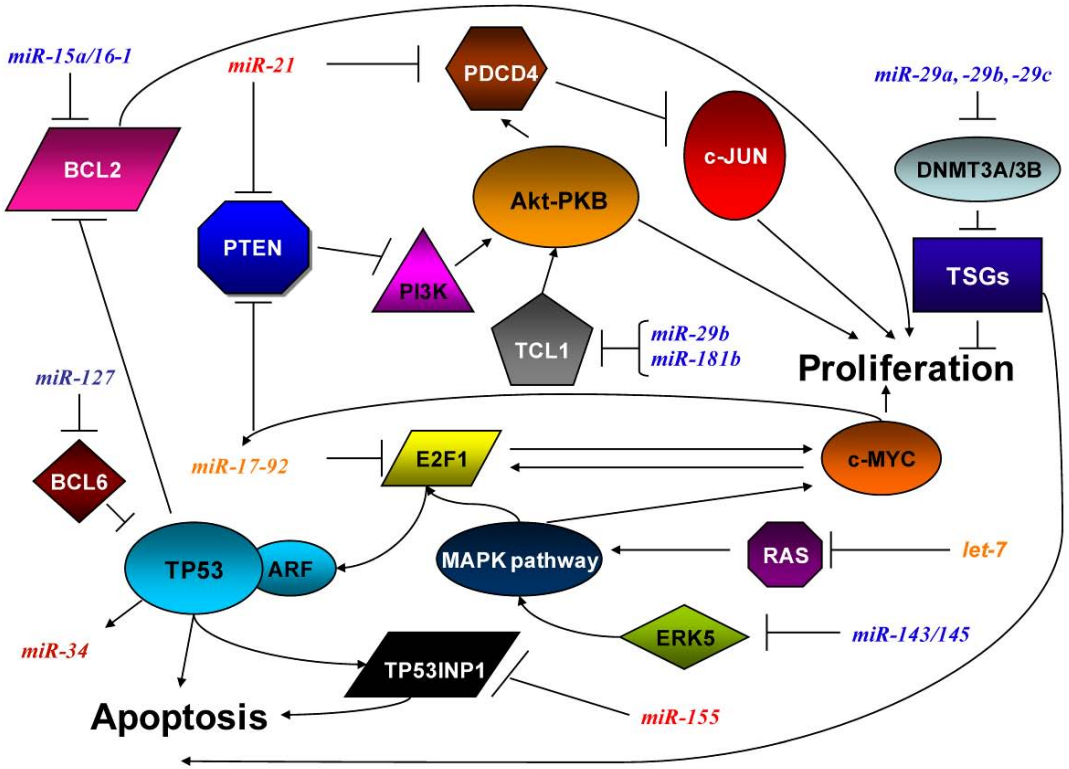
MicroRNA biogenesis and mode of action

- Encoded in the genome
- Transcribed from DNA as pri-microRNA
- Processed into pre-microRNA in the cell nucleus
- In the cytoplasm they are processed into the mature microRNA
- Mature microRNA form complexes with the 3 ´ UTR (untranslated region) of target mRNA and
- Suppress their translation/induce their degradation



The emergence of miRNAs will not make the understanding of regulatory networks easier…

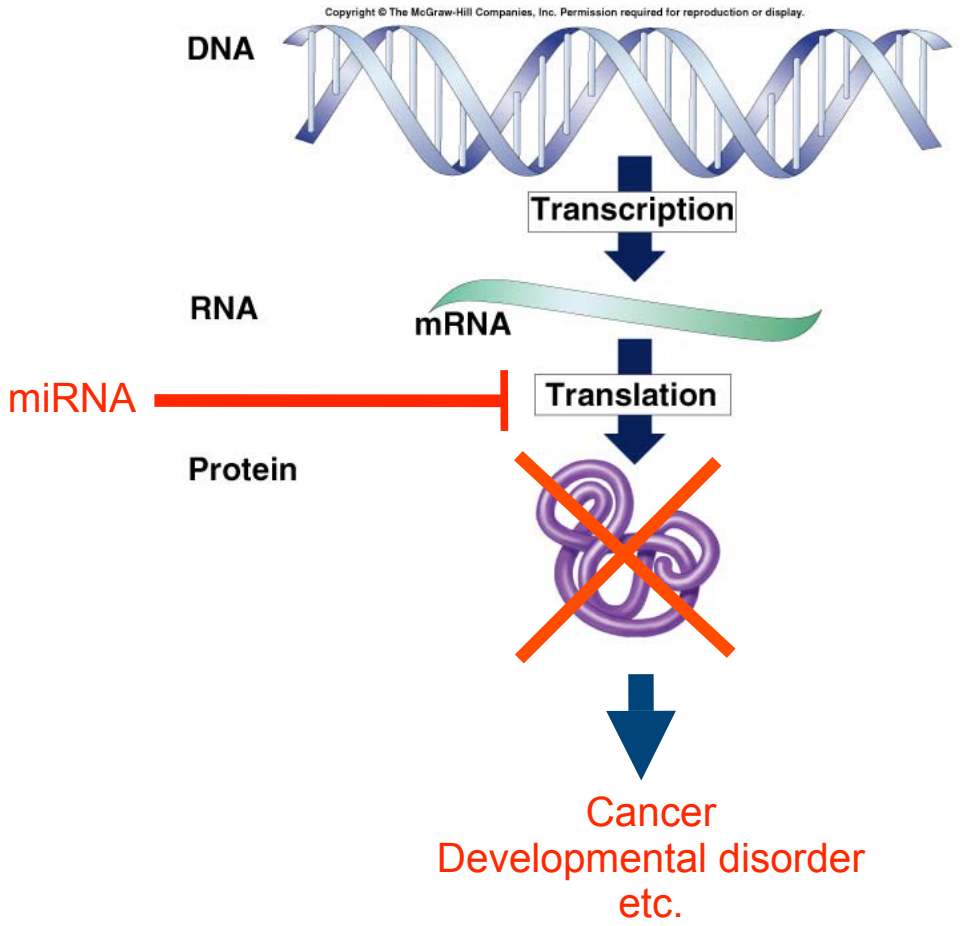
- Each miRNA regulates more than one gene
- Each gene is regulated by more than one miRNA
- Many miRNA targets are transcription factors – miRNAs regulate the regulators
- MiRNA expression is regulated by transcription factors
- The potential regulatory effect of miRNAs is enormous



Modified from George Calin, MD PhD

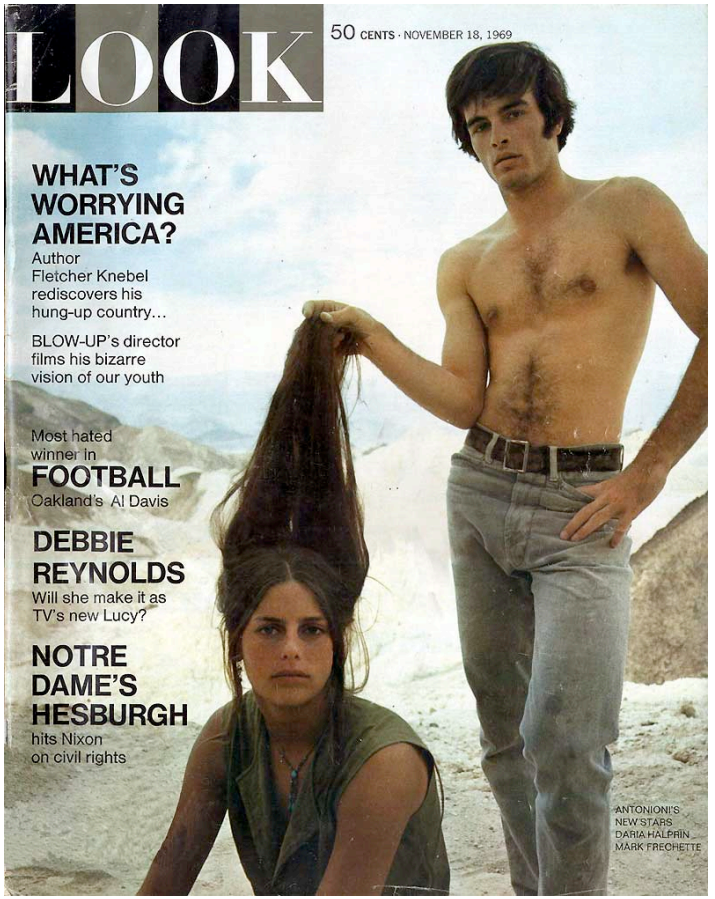
Abnormal miRNA expression can cause diseases

- Altered miRNA exprfession was first implicated in cancers
- MiRNAs may act as
  - tumor suppressors (i.e. miR-16, let-7s)
  - oncogenes (i.e. miR-155, miR-21)
- MiRNAs regulate proliferation, apoptosis and angiogenesis
- In the past few years miRNAs have also been implicated in developmental and metabolic diseases



Why study the skin?

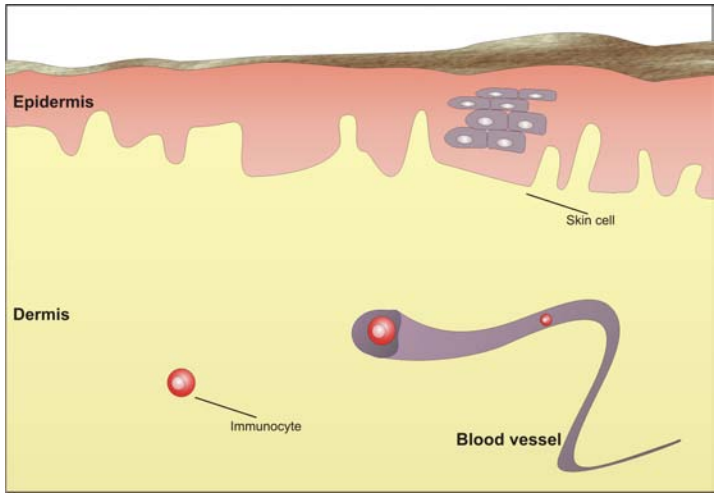
- The largest organ (Surface area 1.5 - 2 m^2; ~9 kg)
- Common and severe diseases
  - Inflammatory diseases (psoriasis, atopic eczema)
  - Tumors (BCC, SCC)
  - Genetic disorders (Xeroderma pigmentosum, ichtyosis)
  - Bullosus
- A handy model for other diseases
  - Easly accesible
  - Skin Immune System (SIS) – Inflammation
  - Carcinogenesis
  - Morphogenesis



Do microRNAs have a role in psoriasis?

- Is there a set of microRNAs that distinguishes healthy skin from psoriasis skin?
- If yes, which are those microRNAs?
- Does microRNA deregulation play a role in psoriasis?

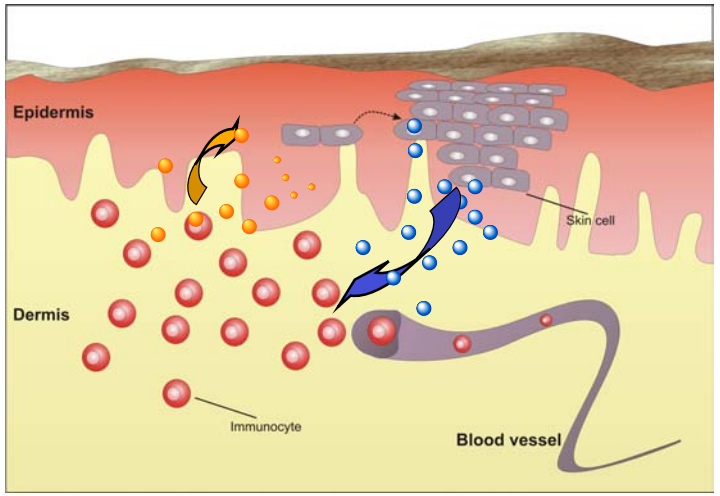
Healthy skin



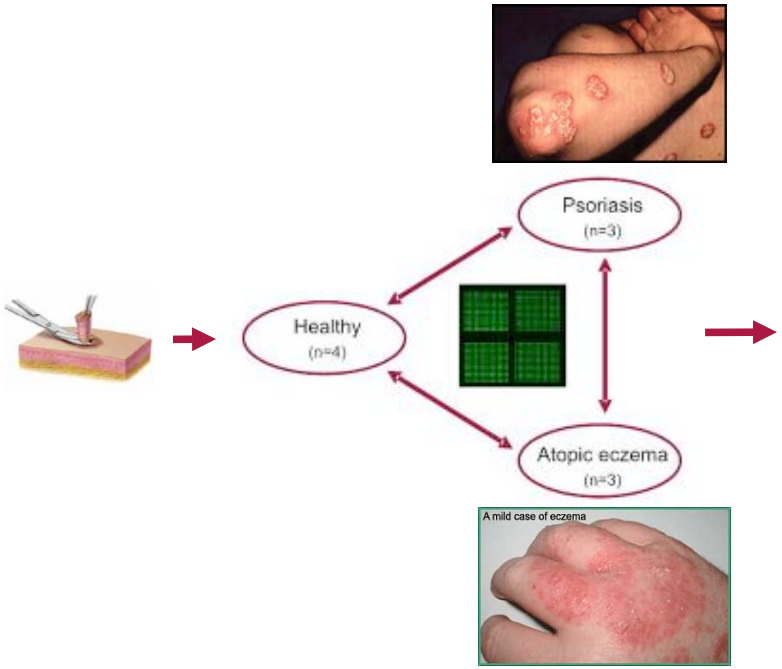
miRNAs?

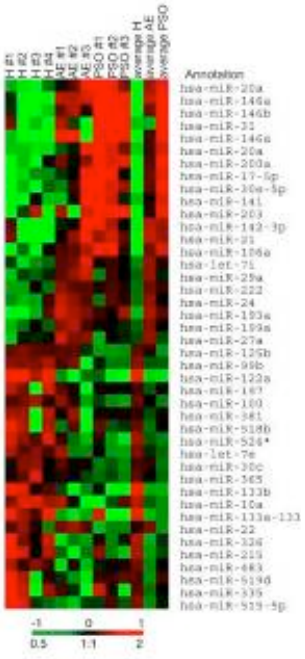
?

Psoriasis



Psoriasis has a specific microRNA expression profile





Psoriasis			Atopic eczema			
miRNA	Score	Fold change	miRNA	Score	Fold change	
miR-146b	4.68	3.31	let-7i	2.50	2.04	UP-REGULATED
miR-20a	4.08	2.90	miR-29a	2.39	1.83	
miR-146a	4.05	3.30	miR-146a	2.35	2.22	
miR-31	3.47	4.69	miR-222	2.22	1.67	
miR-200a	2.85	2.75	miR-24	2.20	1.85	
miR-17-5p	2.67	3.77	miR-193a	2.14	2.27	
miR-30e-5p	2.61	3.61	miR-199a	2.13	1.72	
miR-141	2.26	3.45	miR-27a	2.04	1.72	
miR-203	2.23	5.86	miR-21	2.04	3.26	
miR-142-3p	2.22	2.55	miR-20a	2.02	2.35	
miR-21	2.21	2.51	miR-17-5p	1.82	2.58	
miR-106a	2.16	2.37	miR-106b	1.78	1.72	
miR-125b	-5.31	0.55	miR-122a	-2.75	0.19	DOWN-REGULATED
miR-99b	-3.32	0.58	miR-133a-133b	-2.73	0.28	
miR-122a	-3.09	0.18	miR-326	-2.50	0.39	
miR-197	-2.69	0.64	miR-215	-2.48	0.42	
miR-100	-2.62	0.59	miR-483	-1.89	0.57	
miR-381	-2.60	0.71	miR-519d	-1.86	0.48	
miR-518b	-2.55	0.56	miR-335	-1.78	0.63	
miR-524*	-2.40	0.50	miR-133b	-1.68	0.23	
let-7e	-2.25	0.60	miR-515-5p	-1.57	0.43	
miR-30c	-1.98	0.63				
miR-365	-1.87	0.62				
miR-133b	-1.78	0.22				
miR-10a	-1.78	0.67				
miR-133a-133b	-1.76	0.40				
miR-22	-1.68	0.61				
miR-326	-1.61	0.56				
miR-215	-1.59	0.56				

- Genome-wide analysis of miRNA expression using an array with LNA probes showed that:
  - A set of microRNAs is expressed in human skin
  - Healthy skin, atopic eczema lesion and psoriatic lesions display **distinct microRNA expression profiles**

Sonkoly et al., PloS One, 2007

miR-203 is specifically overexpressed in psoriasis

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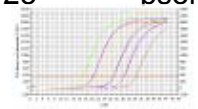
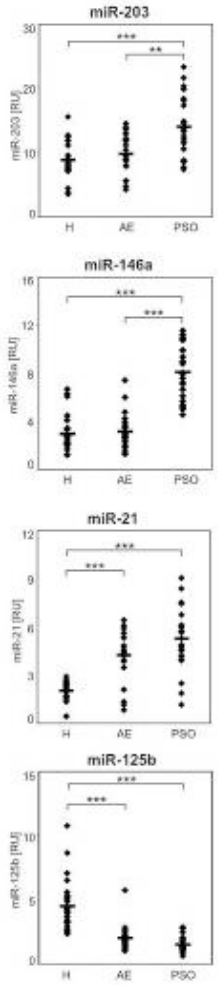
n=28 healthy skin

n=22 atopic eczema

n=25 psoriasis



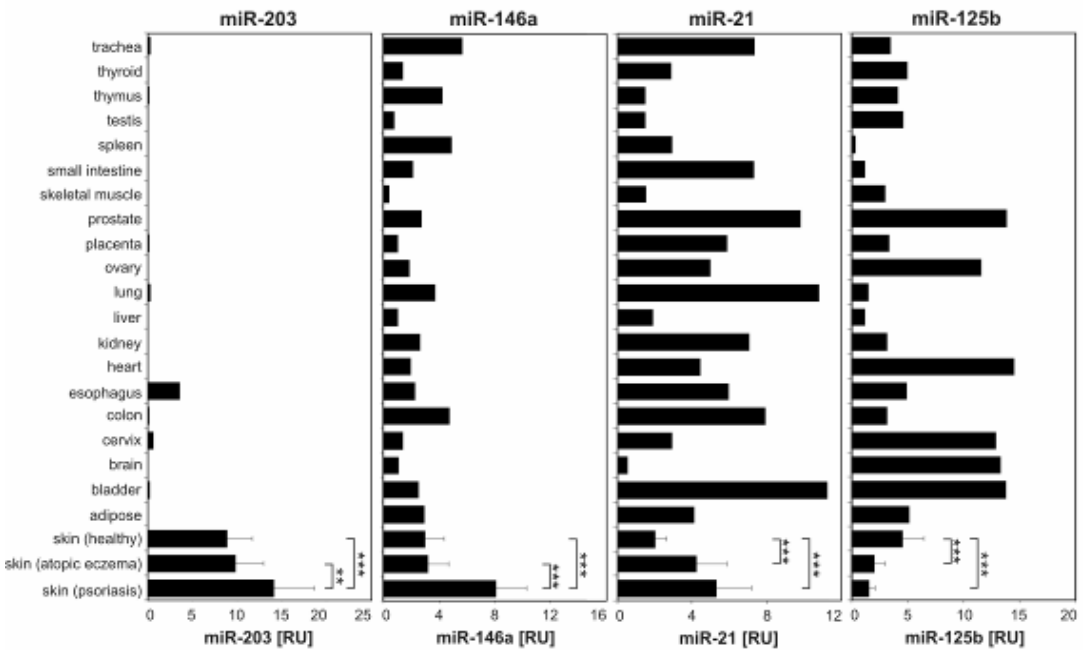


- **miR-203** and miR-146a are overexpressed in psoriasis but not in atopic eczema
- miR-21 is overexpressed and **miR-125b** is suppressed in both diseases
- miR-146 and miR-125b was recently implicated in the regulation of TLR-signaling
- miR-203: potential functions - unknown

Sonkoly et al., PLoS ONE, 2007

miR-203 is a skin-specific microRNA

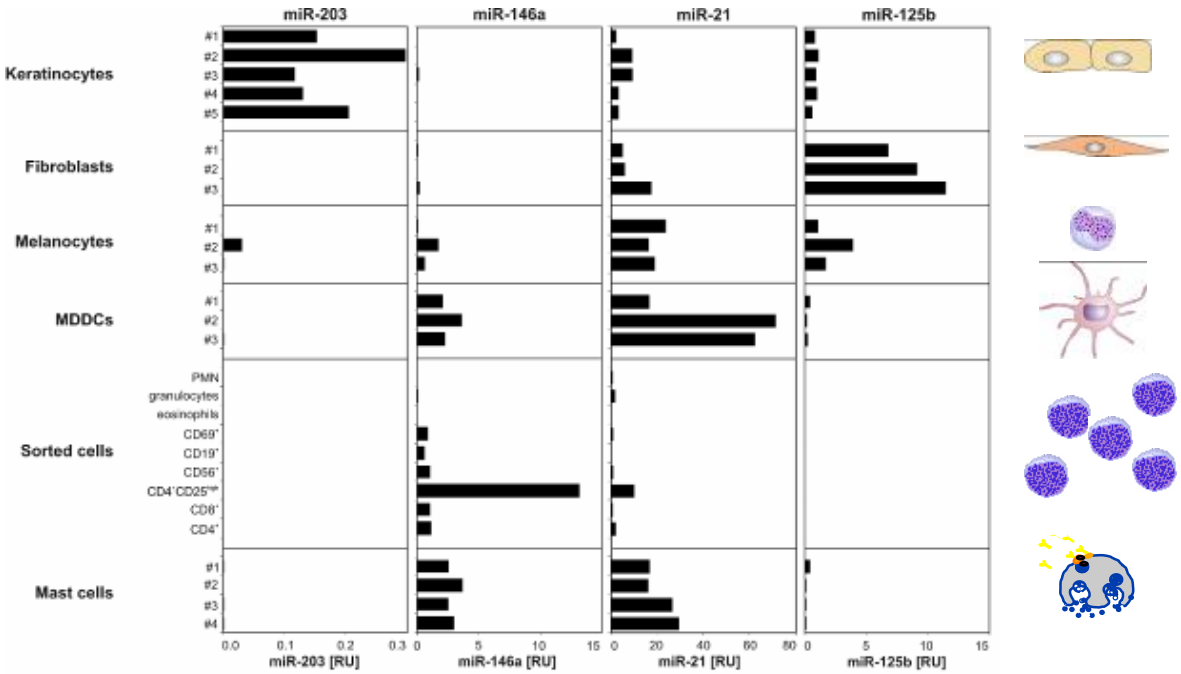
- Highest expression in the skin
- Also expressed in the esophagus
- A role in the differentiation of squamous epithelia?

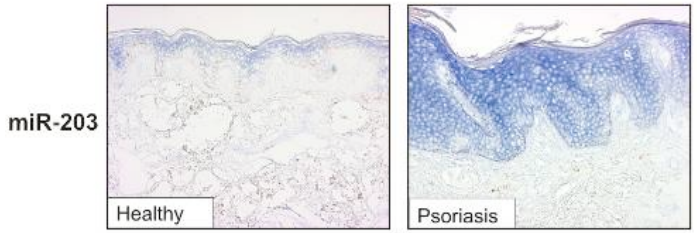


Sonkoly et al., PLoS ONE, 2007

miR-203 is a keratinocyte-specific microRNA

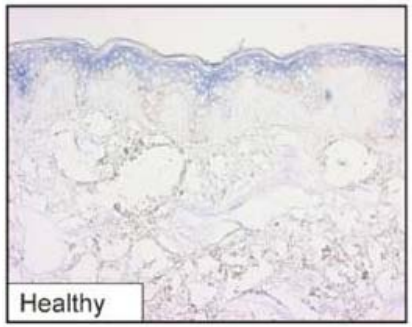
- Keratinocyte-specific expression
- Higher expression in the upper, more differentiated cell layers
- Increased expression in the epidermis of psoriasis lesions

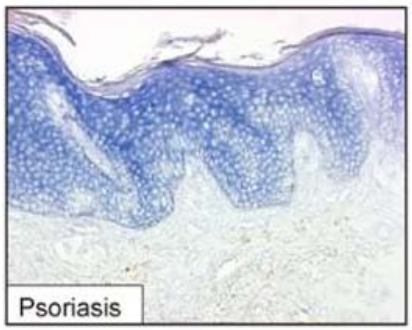


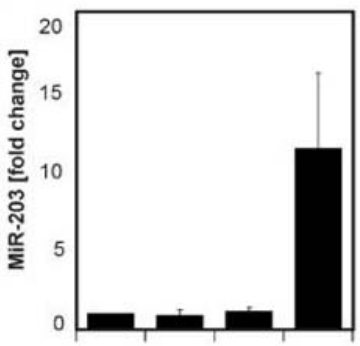


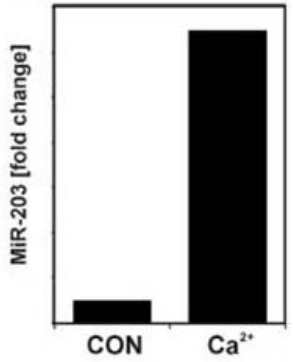
Sonkoly et al., PLoS ONE, 2007

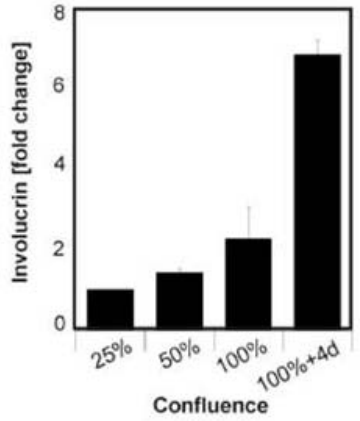
miR-203 is regulated during differentiation



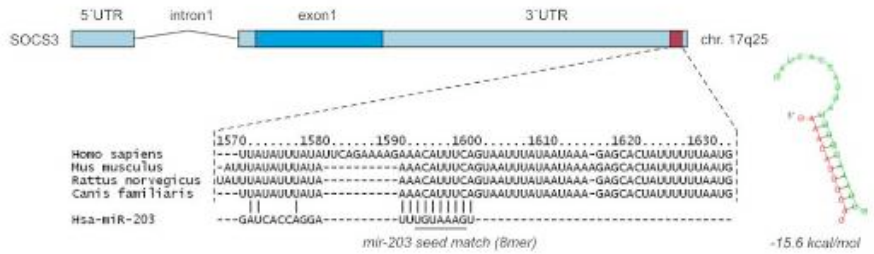


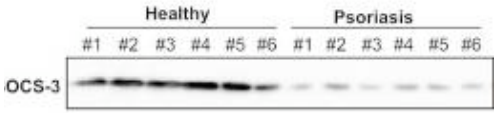


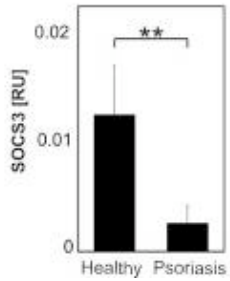




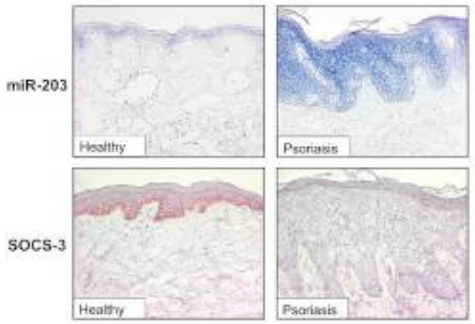
miR-203 targets Suppressor of Cytokine Signaling-3 (SOCS-3)

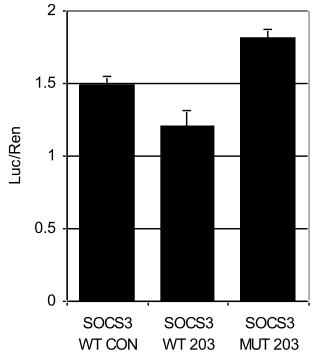






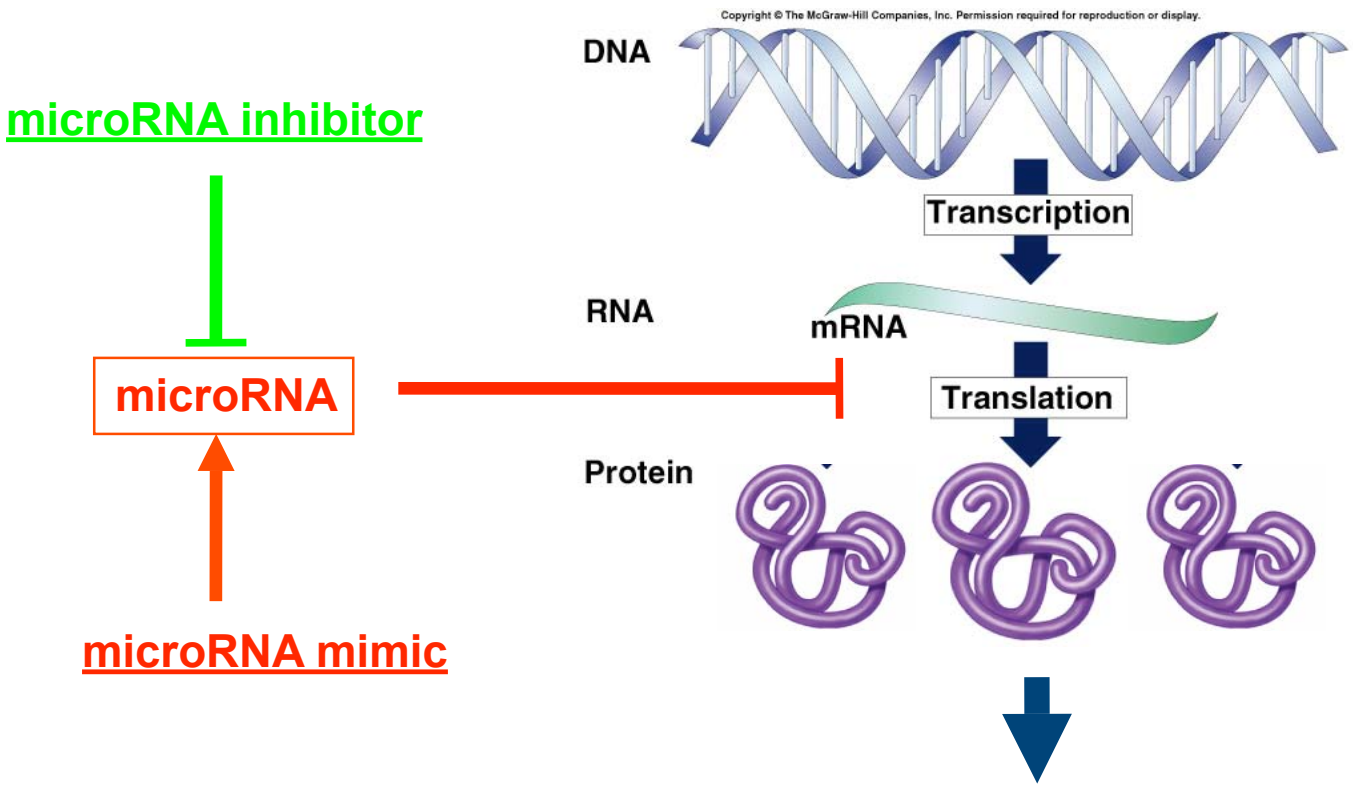
- Predicted binding site for miR-203 in the 3´UTR of SOCS-3 gene
- Suppression of SOCS-3 in psoriasis lesions
- Mutually exclusive staining pattern of miR-203 and SOCS-3 in the epidermis
- Significant repression of the reporter in a luciferase assay
- Suppression of SOCS-3 may result in elongated/increased inflammatory response





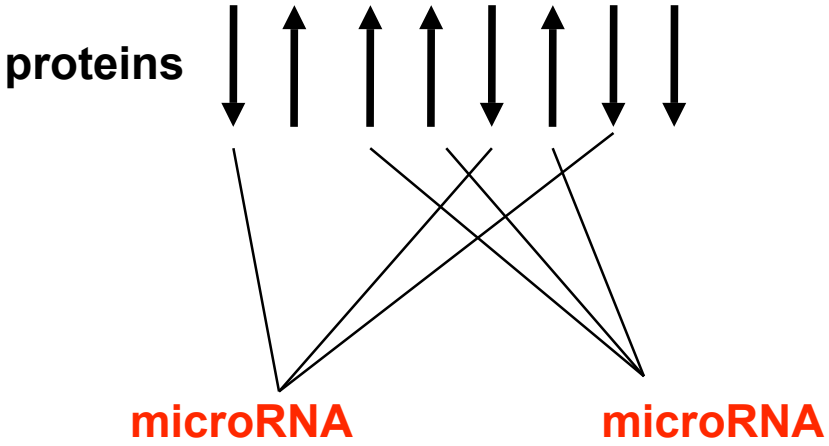
Sonkoly et al., PLoS ONE, 2007

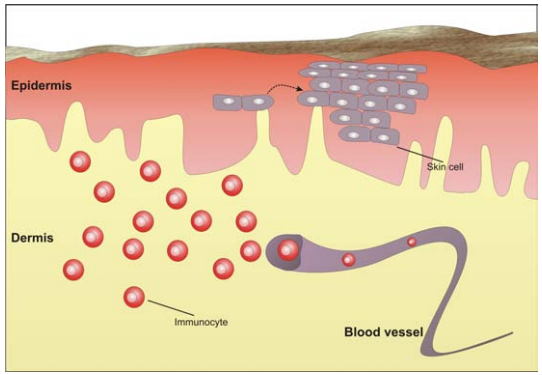
What is the relevance to cosmetics?
- Potential applications



Reduced skin inflammation?

SUMMARY





- microRNA expression patterns distinguish psoriasis from healthy skin and atopic eczema
- miR-203 is a skin- and keratinocyte-specific microRNA
- Its up-regulation in psoriasis is concurrent with the down-regulation of its target, SOCS-3
- a new layer of regulatory mechanisms is involved in the pathogenesis of chronic inflammatory skin diseases

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