MicroRNA-184 - An Ally in Calcium Signalling in the Skin

Adam Richardson[1,2] and Kehinde Ross[1]

[1]School of Pharmacy and Biomolecular Science, Liverpool John Moores University, Liverpool

[2]Current address: Department of Infectious Diseases, King's College London.

Background

The outermost layer of the skin is the epidermis, consisting predominantly of keratinocytes. These cells undergo a process of terminal differentiation as they migrate from the basal layer to the stratum corneum that gives the skin its barrier function. Proteins such as involucrin (IVL) are induced during keratinocyte differentiation, along with the p21 cyclin-dependent kinase inhibitor [1]. In addition, cyclin E has been proposed to drive differentiation through mitotic failure and DNA damage [2].

MicroRNAs (miRNAs) are short noncoding RNAs (18-25 nucleotides) that attenuate post-transcriptional gene output through translational inhibition and destabilization of mRNA transcripts, the latter sustaining steady-state repression [3]. Several miRNAs regulate keratinocyte differentiation and migration, and we previously implicated miR-184 in the cytokine response associated with psoriasis, an inflammatory skin disease [4].

Elevated extracellular Ca^{2+} promotes keratinocyte differentiation and store-operated calcium entry (SOCE) has been implicated in keratinocyte differentiation [5]. Parsimoniously, SOCE is orchestrated by the STIM1 Ca^{2+} sensor on the endoplasmic reticulum and the ORAI family of Ca^{2+} influx channels.

Here, we show that Ca^{2+}-induced differentiation of human primary epidermal keratinocytes (HPEK) upregulates miR-184, and that this occurs in a SOCE-dependent manner. In turn, miR-184 appears to drive HPEK differentiation and migration.

Objectives

The goals of this project were to

- Evaluate the impact of extracellular Ca^{2+} and other HPEK differentiation agents on miR-184 expression
- Assess the effects of SOCE inhibition on miR-184 expression
- Analyse the effects of ectopic miR-184 on keratinocyte differentiation
- Determine the relationship of miR-184 with keratinocyte migration

Methods

The HPEKs were isolated from human foreskin (Liverpool John Moores University Research Ethics Committee approval number 16/PBS/008) or purchased from CellnTec (Bern, Switzerland). Reagents for total RNA extraction, cDNA generation and SYBR Green PCR were purchased from Qiagen. Relative gene expression was determined using the ΔΔCt method. For Western blotting, membranes were probed with relevant primary antibodies at 1:500−1:1000 dilution overnight at 4°C. The miR-184 and control mimics were purchased from GE Dharmacon; the miR-184 inhibitor and control oligonucleotide from Exiqon. Nucleofection was performed using P3 primary cell Nucleofector reagents (Lonza).

References

1.Eckhart, L., et al. (2013). Biochim Biophys Acta 1833, 3471-3480

2.Zanet, J., et al. (2010). PLoS One 5, e15701

3.Bartel, D. P. (2018). Cell 173, 20-51

4.Roberts, J. C., et al. (2013). J Cell Physiol 228, 2314-2323

5.Elsholz, F., et al. (2014). Eur J Dermatol 24, 650-661

Results

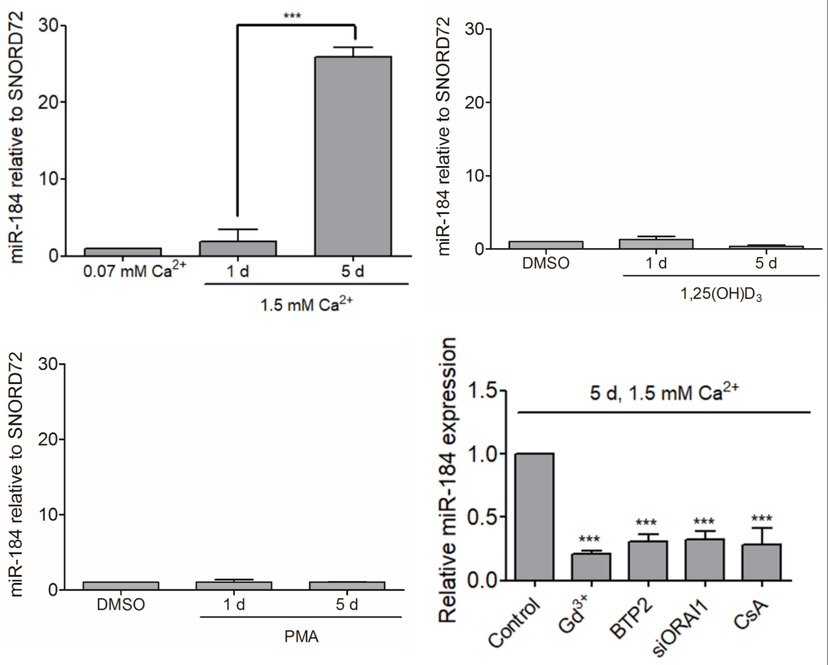

Figure 1: Induction of miR-184 in HPEK by Ca^{2+} but not other differentiation agents: 1.5 mM Ca^{2+}, 100 nM 1, 25-dihydroxyvitamin D3 (1,25-$(OH)_2$D3) or 100 nM phorbol myristate acetate (PMA) for 1 or 5 days (d). For evaluation of the role of SOCE, HPEKs were maintained in 1.5 mM Ca^{2+} for 5 d with or without 1 μM Gd^{3+}, 1 μM BTP2, 100 nM ORAI1-targeting siRNA or 1 CsA μM as indicated. Means +SEM RT-qPCR values from 3 independent experiments normalized to SNORD72; ***, p<0.001

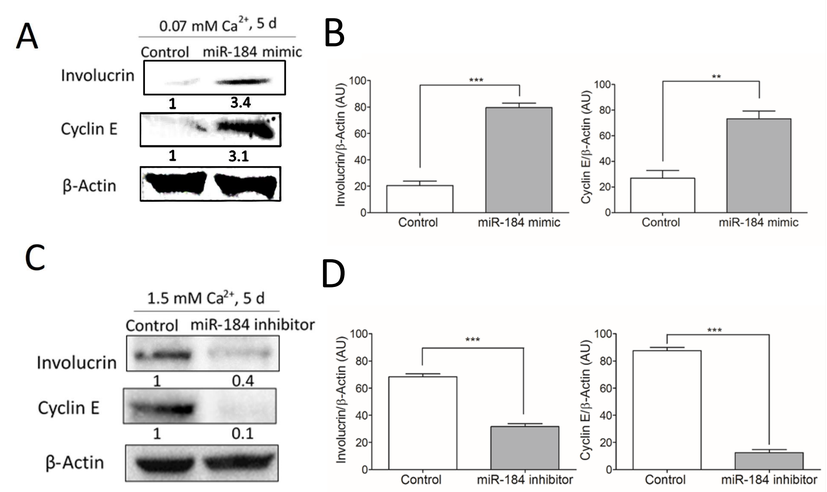

Figure 2: miR-184 induces IVL and cyclin E in HPEK. **(A, C)** Cells loaded with 100 nM miR-184 mimic, miRNA-184 inhibitor or control oligonucleotides by nucleofection were maintained in low Ca^{2+} (0.07 mM) or high Ca^{2+} (1.5 mM) media for 5 days (5 d) as indicated**. (B,D)** Densitometry levels (mean + SEM) relative to β-actin. Data were pooled from 3 independent experiments. ***, p<0.001; **, p<0.01

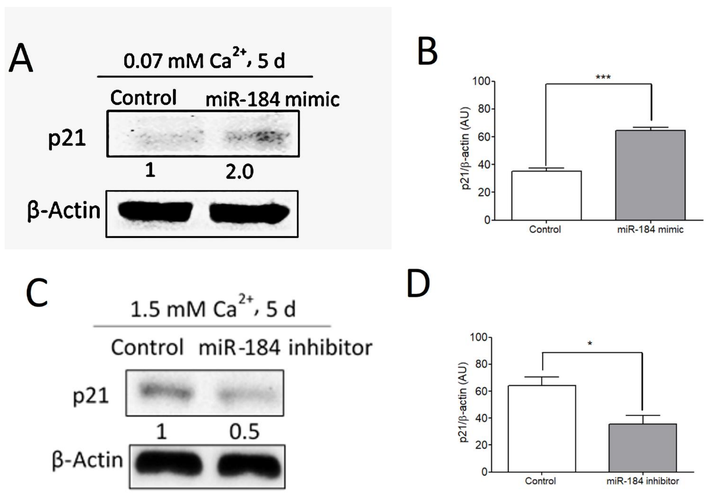

Figure 3: miR-184 induces p21 in HPEK. **(A, C)** Cells loaded with 100 nM miR-184 mimic, miRNA-184 inhibitor or respective control oligonucleotides by nucleofection were maintained in low Ca2+ (0.07 mM) or high Ca^{2+} (1.5 mM) media for 5 days as indicated. **(B, D).** Densitometry levels (mean + SEM) relative to β-actin. Data were pooled from 3 independent experiments. ***, p<0.001; *, p<0.05.

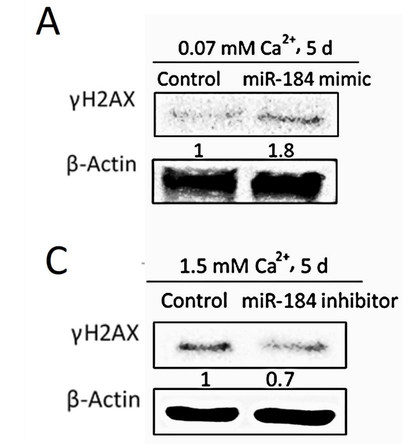

Figure 4: : miR-184 induces p21 in HPEK. **(A, C)** Cells loaded with 100 nM miR-184 mimic, miRNA-184 inhibitor or respective control oligonucleotides by nucleofection were maintained in low Ca^{2+} (0.07 mM) or high Ca^{2+} (1.5 mM) media for 5 days (d) as indicated. **(B, D).** Densitometry levels (mean + SEM) relative to β-actin. Data were pooled from 3 independent experiments. ***, p<0.001; *, p<0.05.

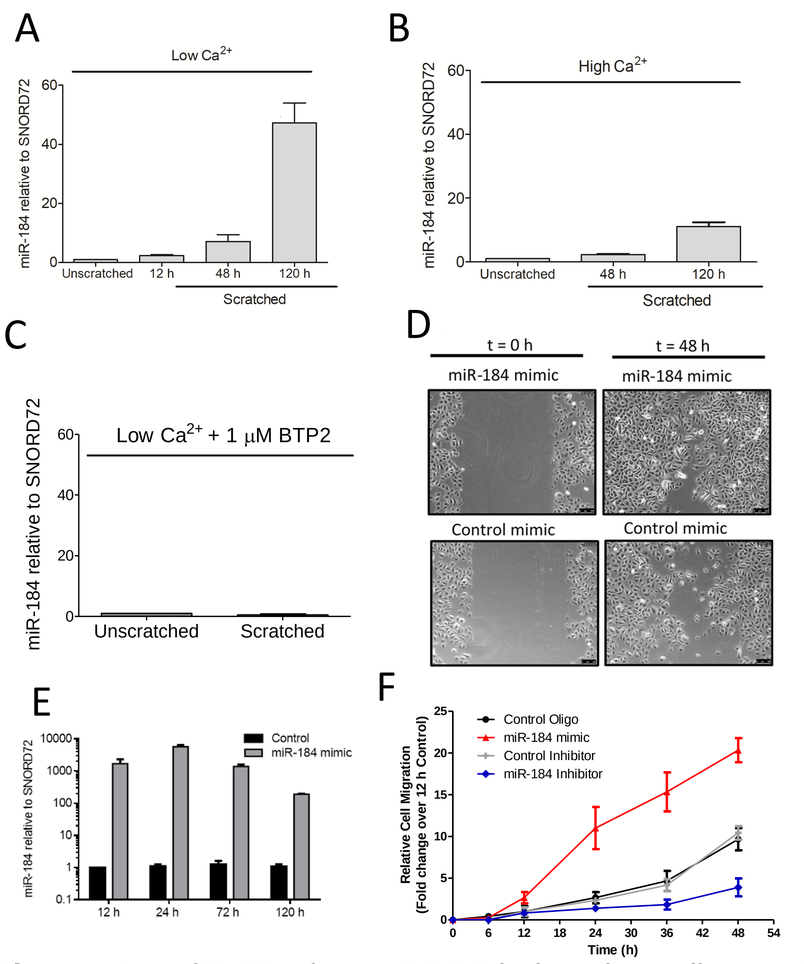

Figure 5: miR-184 is a SOCE-induced mediator of keratinocyte migration. **(A,B)** HPEK monolayers were scratched in a lattice pattern and harvested after maintenance in low (0.07 mM) or high (1.5 mM) Ca^{2+}. Normalised miR-184 expression is depicted relative to corresponding unscratched controls. **(C)**The SOCE blocker BTP2 abolished scratch-dependent induction of miR-184. **(D)** Micrograph of scratched HPEK monolayers depicting enhanced migration is cells loaded with 100 pmol miR-184 mimic. **(E)** Confirmation of elevated miR-184 expression in HPEK loaded with miR-184 mimic. **(F)** Relative migration rates for scratched HPEK loaded with miR-184 mimic, miR-184 inhibitor and controls, respectively.

Conclusions

- miR-184 is upregulated during Ca^{2+}-dependent differentiation of human epidermal keratinocytes
- miR-184 induction occurs in a SOCE-dependent manner in differentiating keratinocytes under high Ca^{2+} conditions and in wounded keratinocyte monolayers maintained in low Ca^{2+}
- Ectopic miR-184 promotes keratinocyte differentiation and migration

Acknowledgement

We thank the British Skin Foundation for generously funding the study.