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MicroRNA-184 and its lncRNA sponge UCA1 are induced in wounded keratinocytes in a store-operated calcium entry-dependent manner

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TO THE EDITOR

There remains a significant need for therapeutic interventions for wound healing as the clinical and socioeconomic challenges presented by the ageing population and associated rise in non-healing wounds persist, exerting enormous pressure on health services [1].

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MicroRNAs (miRNAs) are short ≈22 nucleotide non-coding RNA molecules that fine-tune gene expression by degradation of mRNA targets [2]. Several miRNAs promote keratinocyte migration, including miR-21, miR-31 and miR-132 (reviewed in [3]), hence miRNA-dependent migration is emerging as a framework for wound healing.

Depletion of calcium (Ca^{2+}) from the endoplasmic reticulum (ER) is coupled to extracellular Ca^{2+} influx from the milieu. This store-operated Ca^{2+} entry (SOCE) is mediated by STIM1, which senses the drop in ER Ca^{2+} levels and aggregates to activate ORAI1, the predominant SOCE channel [4]. However, relationships between SOCE and miRNAs have received little attention.

In previous work, miR-184 was largely absent from proliferating human primary epidermal keratinocytes (HPEK; CellnTec, Switzerland) under low Ca^{2+} culture conditions [5],. However, miR-184 was recently observed in the suprabasal mouse epidermis, though it was low or absent in e basal layers [6]. Given that keratinocyte migration is inherent to epidermal stratification, we hypothesized that miR-184 induction may be associated with keratinocyte migration during wound healing.

We performed high-density scratch assays on HPEK and examined miR-184 expression using reverse-transcription quantitative PCR (RT-qPCR) reagents (Qiagen, UK). In scratched HPEK monolayers, miR-184 rose 2-, 7-and 50-fold after 12 h, 48 h and 120 h, respectively (Fig.1a). Interestingly, miR-184 was also induced when scratched HPEK were differentiating in high (1.5 mM) Ca^{2+} medium, 2- and 11-fold at 48 h and 120 h, respectively (Fig. 1b). Note that miR-184 in unscratched HPEK following 120 h high Ca^{2+} was 5-fold higher than in the low Ca^{2+} counterpart. We focussed on proliferating cells and determined whether SOCE contributed to miR-184 induction. Incubation of scratched HPEK monolayers with SOCE inhibitors (1 μM Gd^{3+} or 1 μM BTP2, added 1 h before wounding) abolished miR-184 upregulation (data not shown).

Long non-coding RNAs (lncRNAs) are a heterogeneous group of ncRNAs $\geq$ 200 nucleotides long with diverse mechanisms of action [7]. Growing evidence suggests lncRNAs can function as competing endogenous RNAs (ceRNAs) to sequester and limit miRNAs, though the ceRNA hypothesis has been challenged. The lncRNA UCA1 (urothelial carcinoma associated 1) may be a ceRNA for miR-184, possessing four miR-184-binding sites [8].

We therefore examined UCA1 expression in HPEK samples. An 11-fold induction of UCA1 was observed in scratched compared to unscratched monolayers (Fig.1c). Blockade of SOCE with Gd^{3+} or BTP2 reduced UCA1 induction by ~50% (data not shown). Thus UCA1 appears to be co-induced with miR-184 in wounded HPEK and UCA1 upregulation was at least partly dependent on SOCE.

We evaluated the impact of miR-184 on HPEK migration by transfecting 100 nM miR-184 mimic (GE Healthcare, UK) using nucleofection. Levels of miR-184 levels were 6000-fold higher 24 h post-transfection, dropping to ~1000- then 200-fold after 72 h and 120 h, respectively (Fig, 1d). A single scratch was made on transfected monolayers as they approached confluence. Exogenous miR-184 increased the rate of HPEK migration compared to control cells loaded with a non-targeting oligonucleotide (Fig. 1e), with wound closure largely complete by 48 h. In contrast, blockade of miR-184 using 100 nM of a locked nucleic acid (LNA) inhibitor (Exiqon, Denmark) impaired HPEK migration compared to cells loaded with a non-targeting control LNA (Fig. 1f). Linear regression of the 12-36 h period from the relative migration rates (Fig. 1g) yielded migration indices of 0.53±0.11 per hour for miR-184-loaded HPEK, compared to 0.15±0.05, 0.13±0.03 and 0.04±0.02 per hour for cells loaded with control mimic, control inhibitor and miR-184 inhibitor, respectively. We

confirmed the miR-184 inhibitor downregulated miR-184 (Fig. 1h). Hence, elevation of miR-184 appeared to enhance keratinocyte migration 3-fold while miR-184 inhibition decelerated migration 3-fold.

Our results indicate for the first time that wounding leads to *de novo* miR-184 expression in proliferating keratinocytes, miR-184 upregulation in differentiating keratinocytes and induction of UCA1. Elevation of miR-184, and to a lesser extent UCA1, depended on SOCE even though extracellular Ca^{2+} was low. The impact of SOCE on miRNA and lncRNA expression thus requires further investigation. Exogenous miR-184 promoted keratinocyte migration, adding miR-184 to the subset of miRNAs implicated in re-epithelialisation.

Limitations of our study include the absence of transcriptome-wide miR-184 target identification linked to keratinocyte migration. Additionally, we have not shown direct UCA1 function as a miR-184 sponge in keratinocytes. It will be interesting to fully define the axis from wounding to UCA1 induction and the links between UCA1 and miR-184-regulated migration.

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REFERENCES

1 Whittam AJ, Maan ZN, Duscher D *et al.* Challenges and Opportunities in Drug Delivery for Wound Healing. *Adv Wound Care (New Rochelle)* 2016; **5**: 79-88.

2 Eichhorn SW, Guo H, McGeary SE *et al.* mRNA Destabilization Is the Dominant Effect of Mammalian MicroRNAs by the Time Substantial Repression Ensues. *Mol Cell* 2014; **56**: 104-15.

3 Ross K. Towards topical microRNA-directed therapy for epidermal disorders. *J Control Release* 2018; **269**: 136-47.

4 Gudlur A, Hogan PG. The STIM-Orai Pathway: Orai, the Pore-Forming Subunit of the CRAC Channel. *Adv Exp Med Biol* 2017; **993**: 39-57.

5 Roberts JC, Warren RB, Griffiths CE *et al.* Expression of microRNA-184 in keratinocytes represses argonaute 2. *J Cell Physiol* 2013; **228**: 2314-23.

6 Nagosa S, Leesch F, Putin D *et al.* microRNA-184 Induces a Commitment Switch to Epidermal Differentiation. *Stem cell reports* 2017; **9**: 1991-2004.

7 Dykes IM, Emanueli C. Transcriptional and Post-transcriptional Gene Regulation by Long Non-coding RNA. *Genomics Proteomics Bioinformatics* 2017; **15**: 177-86.

8 Zhou Y, Wang X, Zhang J *et al.* Artesunate suppresses the viability and mobility of prostate cancer cells through UCA1, the sponge of miR-184. *Oncotarget* 2017; **8**: 18260-70.

FIGURE LEGEND

Figure 1: miR-184 as a SOCE-induced mediator of keratinocyte migration. Confluent HPEK monolayers were scratched in a lattice pattern at 0.5 cm intervals and harvested after maintenance in low (0.07 mM) or high (1.5 mM) Ca^{2+} media as indicated. (a,b) miR-184 expression was evaluated by RT-qPCR normalised to SNORD72 and depicted relative to corresponding unscratched controls. (c) Expression of the miR-184 sponge UCA1 was examined by RT-qPCR and normalised to GAPDH. (d) HPEK loaded with 100 nM of miR-184 mimic (*grey*) or control non-targeting mimic (*black*) were cultured in 0.07 mM Ca^{2+} medium for the indicated duration. miR-184 expression was normalised to SNORD72 and depicted relative to 12 h control. (e,f) Cells were loaded with 100 nM of miR-184 mimic or miR-184 inhibitor as indicated. Monolayers were scratched upon approaching confluence and monitored at 30 min intervals for 48 h. *Scale bar* = 100 μm. (g) The scratch was sectioned into three and migration rates were estimated from the number of cells populating the inner scratched region and depicted relative to 12 h. (h) HPEK loaded with 100 nM of miR-184

inhibitor (*grey*) or control non-targeting inhibitor (*black*) were cultured in 0.07 mM Ca^{2+} medium for the indicated duration. miR-184 expression was normalised to SNORD72 and depicted relative to unscratched control. Data for all graphs were pooled from 3 independent experiments. Statistical analyses were performed using Student's *t* test were indicated, **, $p<0.05$ was considered significant.