The ATP-dependent chromatin remodeler BRG1 controls epidermal keratinocytes migration during human cutaneous wound healing

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Background

- SWI/SNF ATP-dependent chromatin remodeling complexes are large multi-subunit complexes capable of regulating nuclear processes, including gene transcription.
- SWI/SNF complexes consist of an ATPase subunit (BRG1 or hBRM), several core subunits and a multitude of accessory subunits.
- BRG1 has previously been shown to be required for terminal epidermal differentiation in both mice and humans (1, 2)
- Brg1 is a direct transcription target of p63 in epidermal progenitor cells and is required for the relocation of the EDC genomic locus into nuclear interior where the environment facilitates gene transcription (3)
- The role for BRG1 in epidermal development has been well established, however the role in human cutaneous wound healing remains unknown

Hypothesis

- We hypothesize that SWI/SNF complex is required for efficient skin wound healing in humans.

Methods

- *Ex vivo* wound healing models were established from female scalp (donors aged 50-67 yrs (n=3)) and treated them with either non-targeting or SMARCA4 siRNA and measured the length and thickness of the migrating epithelia 3 and 5 days later.
- Scratch mediated wound healing assay was completed on keratinocytes isolated from both male and female donors aged 29-67 yrs (n=7) and treated with non-targeting or SMARCA4 siRNA for 48 hours prior to scratching, the rate or wound closure was then measured.
- RNA lysates were collected from the scratch assay at 1, 24 and 48 hours and analysed using the Agilent microarray platform, key gene changes were further analysed using DAVID GO term enrichment.

Results

BRG1 is significantly upregulated in the cutaneous wound hyper-proliferative and migrating epithelia

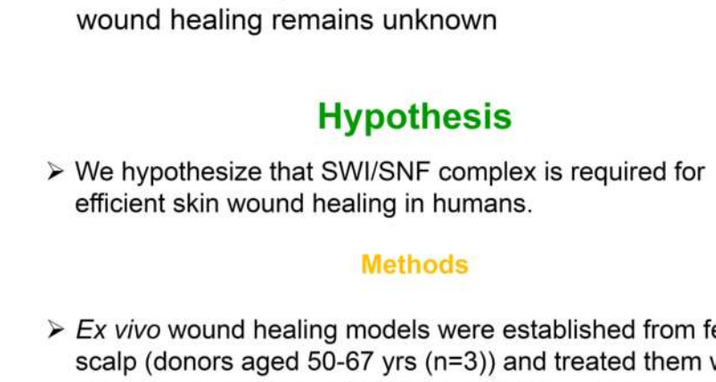


Figure 1. IF images demonstrating the distribution of BRG1 protein in the distal epidermis **(A)** and the wound hyper-proliferative and migrating epithelia **(B)**. **(C)** Comparison of the fluorescence intensity of distal and wound hyper-proliferative and migrating epithelia. Mean and + S.D. shown N=3 donors, n=3 sections per donor. Mann-Whitney U-test *P = 0.0003.

SMARCA4/BRG1 siRNA significantly inhibits BRG1 expression in the human ex vivo cutaneous wound healing model

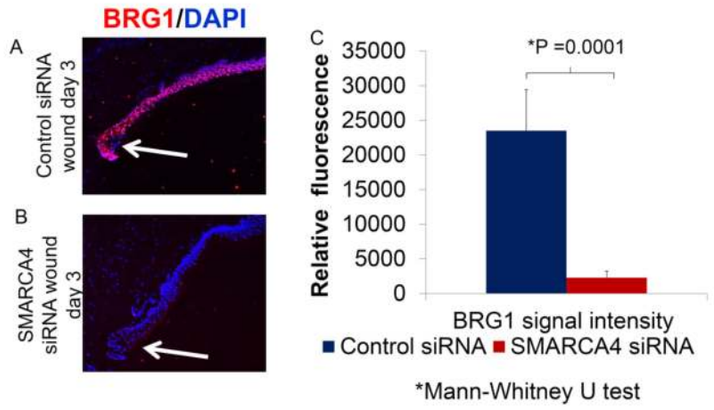


Figure 2. IF images demonstrating the distribution of BRG1 protein in the wound hyper-proliferative and migrating epithelia treated with either control non-targetting siRNA **(A)** or SMARCA4 siRNA **(B)**. **(C)** Comparison of the fluorescence intensity of wound hyper-proliferative and migrating epithelia treated with either control siRNA or SMARCA4 siRNA. Mean and + S.D. shown N=3 donors, n=3 sections per donor. Mann-Whitney U-test *P = 0.0001

SMARCA4/BRG1 suppression results in a significant delay in wound healing

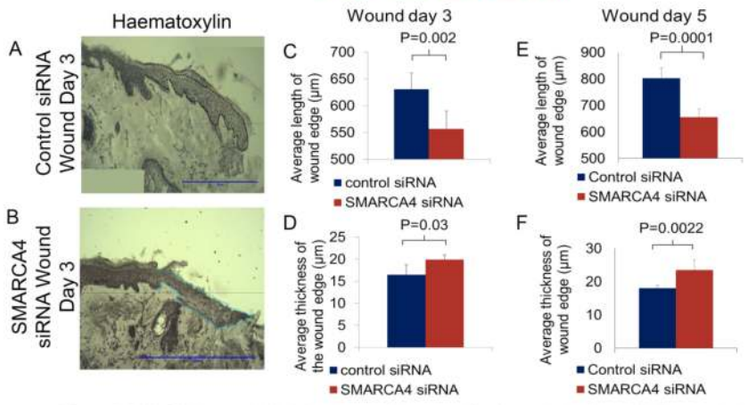


Figure 3. Brightfield images highlighting the wound hyper-proliferative and migrating epithelia treated with either control non-targetting siRNA **(A)** or SMARCA4 siRNA **(B)**. Comparison of wound characteristics (length **(C, F)** or thickness **(D, F)**) of wound hyper-proliferative and migrating epithelia treated with either control siRNA or SMARCA4 siRNA at both 3 and 5 days. Mean and + S.D. shown, N=3 donors, n=3 sections per donor, at least 20 measurements per section. Mann-Whitney U-test

SMARCA4 siRNA significantly inhibits BRG1 expression in normal human epidermal keratinocytes (NHEKs)

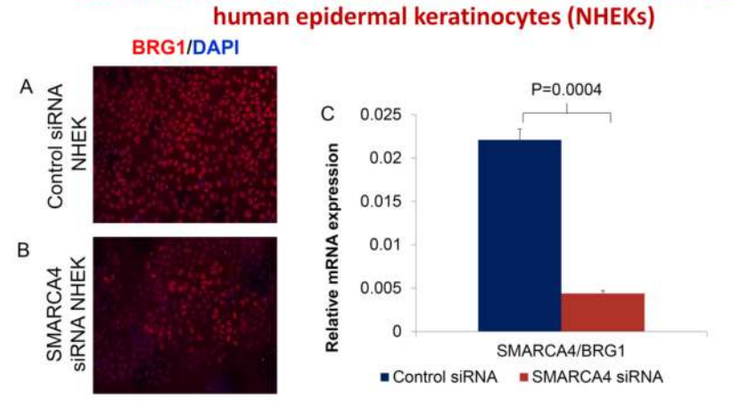


Figure 4. IF images demonstrating the distribution of BRG1 protein in NHEKs treated with either control siRNA **(A)** SMARCA4 siRNA **(B)**. **(C)** Comparison of BRG1 mRNA expression of harvested NHEKs after treatment with either control siRNA or SMARCA4 siRNA. Mean and + S.D. shown N=6 donors, n=3 wells per treatment per donor. Student's T-Test.

SMARCA4/BRG1 suppression has no effect on proliferation in NHEKs

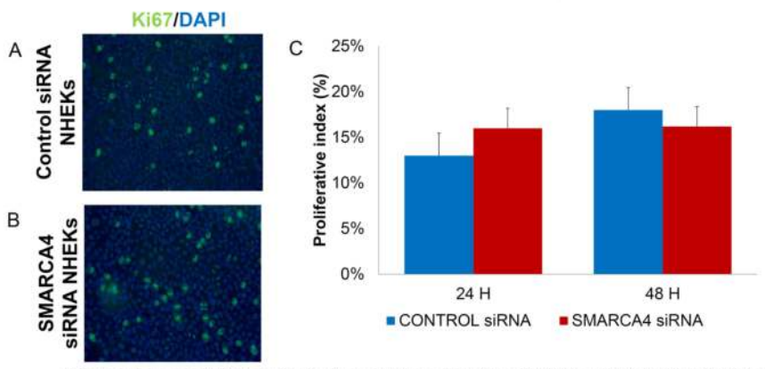


Figure 5. IF images of Ki67 staining in either control non-targetting siRNA **(A)** or SMARCA4 **(B)** siRNA treated NHEKs. **(C)** Proliferation index (Ki67 positive cells/Total cells) of either control siRNA or SMARCA4 siRNA treated NHEKs at 24 and 48 hours. Mean and + S.D. shown N=6 donors, n=3 wells per group per donor. Student's T-test

SMARCA4/BRG1 suppression results in a significant delay in scratch wound closure in HPEK healing

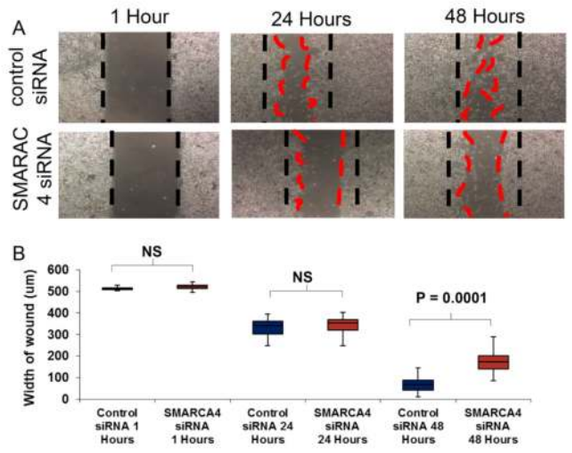
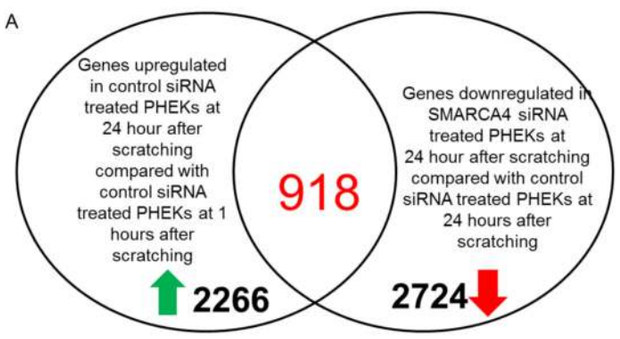


Figure 6. (A) Representative images of scratch mediated wound healing assay at 1, 24 and 48 hours post scratching comparing control non-targetting siRNA or SMARCA4 siRNA treated NHEKs, Black dashed line deonte original scratch while red dashed line denotes cellular migration. **(B)** Box and whisker plots measuring rate of migration comparing control or SMARCA4 siRNA treated NHEKs at 1, 24 or 48 hours post scratching. 1st, 3rd quartiles, median, maximum and minimum values shown, N=6 donors, n=3 wells per group per donor. Mann-Whitney U test.

SWI/SNF complexes regulates cellular mobility during wound healing response



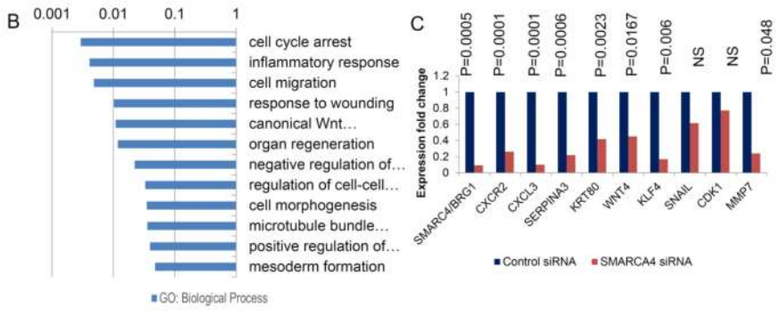


Figure 7 (A) Venn diagram indicating the total number of genes upregulated in control siRNA PHEKs at 24 hours after scratching (2266), compared with number of genes downregulated in SMARCA4 siRNA at 24 hours after scratching (2724), the overlap are genes upregulated in at 24 hours after scratching in control cells, but downregulated at 24 hours after scratching in SMARCA4 suppressed cells (916) determined using Agilent Microarray Transcriptomics. **(B)** GO term enrichment of the 918 shared genes identified using DAVID bioinformatic resources. The bars represent the log10 p-values of each category. **(C)** RT-qPCR validation of several key genes highlighted from the GO enrichment analysis, mean and +S.D. shown, N=3 donors, experiments run in triplicate

Conclusions

- BRG1 is upregulated in the hyper-proliferating and migrating epithelia in the cutaneous wound.
- The SWI/SNF complex controls epidermal keratinocyte migration without affecting their proliferation and apoptosis during skin wound healing
- The SWI/SNF complex control changes in transcription programmes associated with epithelial cell cytoskeletal remodelling and cell mobility during skin wound healing

References

1) Indra et al., *Development 2005.*
2) Bao et al., *Genome Biology 2015.*
3) Mardaryev et al., *Development 2014)*