ATP-dependent chromatin remodeller BRG1 modulates human epidermal keratinocyte migration during cutaneous wound healing

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Background

>>> ATP-dependent SWI/SNF chromatin remodelling complexes are large multi-subunit complexes controlling nuclear processes, including gene transcription

>>> SWI/SNF complexes remodel chromatin structure using the energy of ATP hydrolysis to change nucleosome conformation, facilitate nucleosome sliding, nucleosome ejection and histone variant switching

>>> The SWI/SNF complexes contain either BRG1 or BRM as the core ATPase in addition to 9-11 other subunites

>>> BRG1 is required for terminal keratinocyte differentiation in mice and humans and for the hair follicle epithelial stem cell maintenance and activation during hair cycle and wound associated skin regeneration in mice

>>>The role of BRG1 in human wound healing and hair growth remains largely unknown

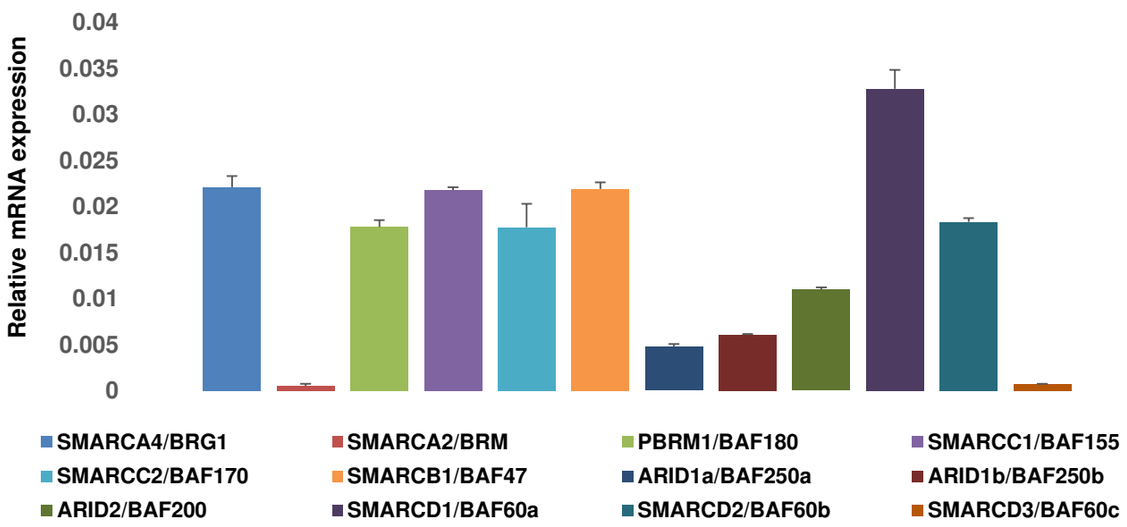
Objectives

>>> Determine the role of BRG1 in human epidermal keratinocytes proliferation, survival and migration during skin wound healing

>>>Define the role of BRG1 in control of gene expression program changes in human epidermal keratinocyte during skin wound healing

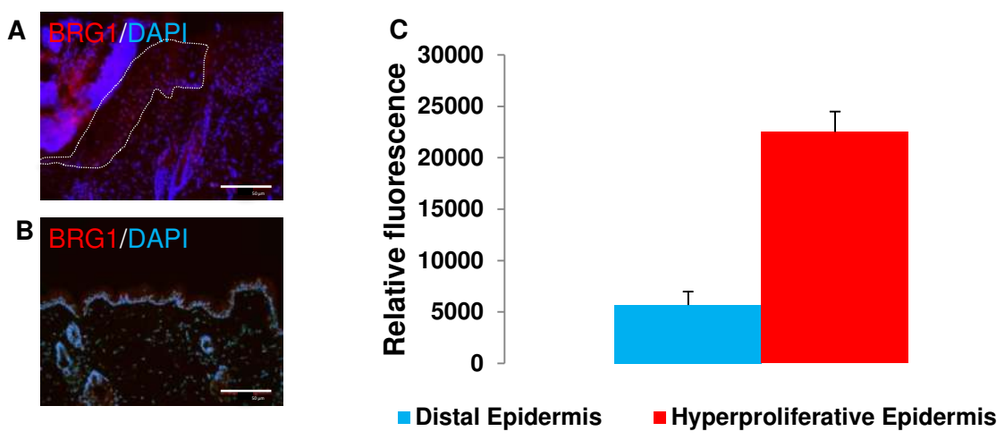
Results

Figure 1. Most genes encoding SWI/SNF subunits are expressed in epidermal keratinocytes



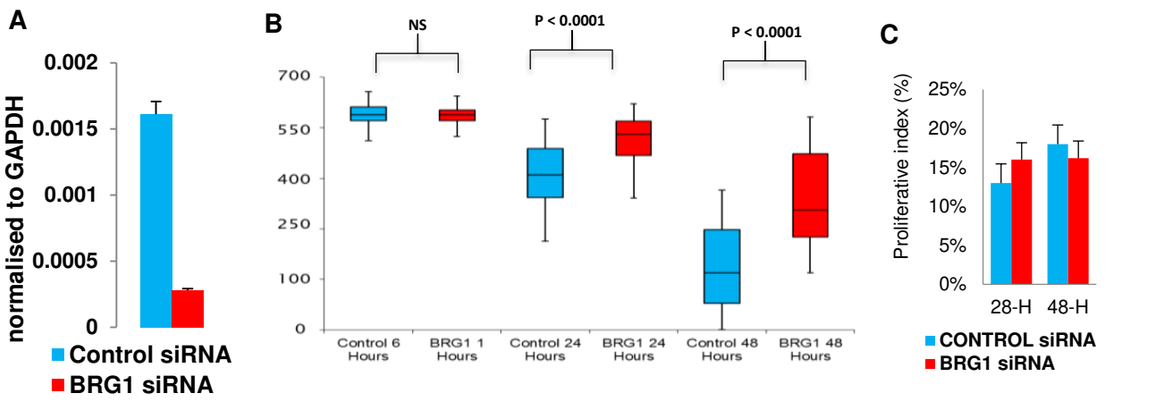
qRT-PCR analysis of RNA isolated from the primary human epidermal keratinocytes (PHEK) with primers specific for indicated genes. The data is normalized to the GAPDH as a reference gene. Mean and standard deviation are shown from three independent biological samples with qRT-PCR reactions run in duplicate.

Figure 2. BRG1 protein expression is increased the hyperproliferative epithelium of skin wound in comparison to distal unaffected epidermis



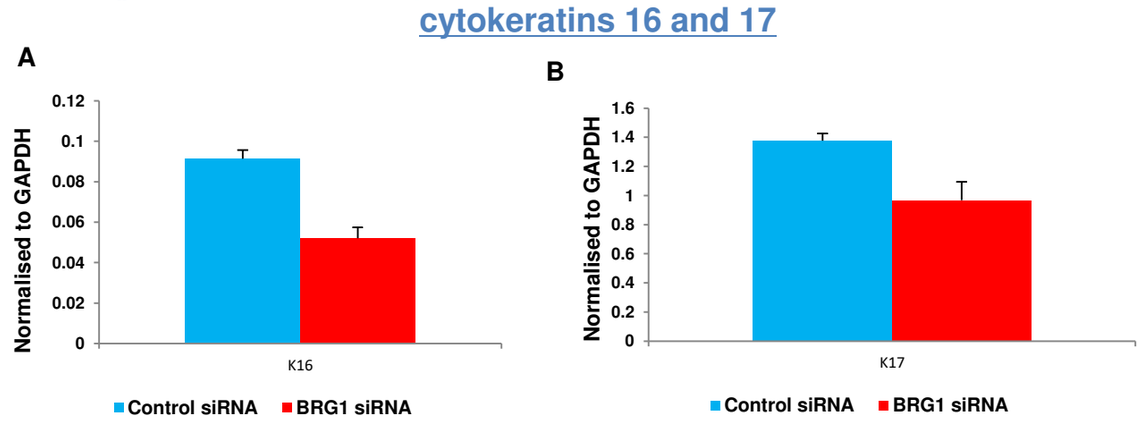
A, B Immuno-fluorescent analysis of Brg1 protein expression in the mouse wound hyperproliferative epidermis, three days after wounding (A) and distal unwounded epidermis (B) dotted line denotes HPE. C. Quanitative analysis of the immuno-fluorescent signals shown in A and B.

Figure 3. BRG1 controls migration, but not proliferation of HaCat keratinocyte HaCat keratinocytes after scratch associated wounding in vitro



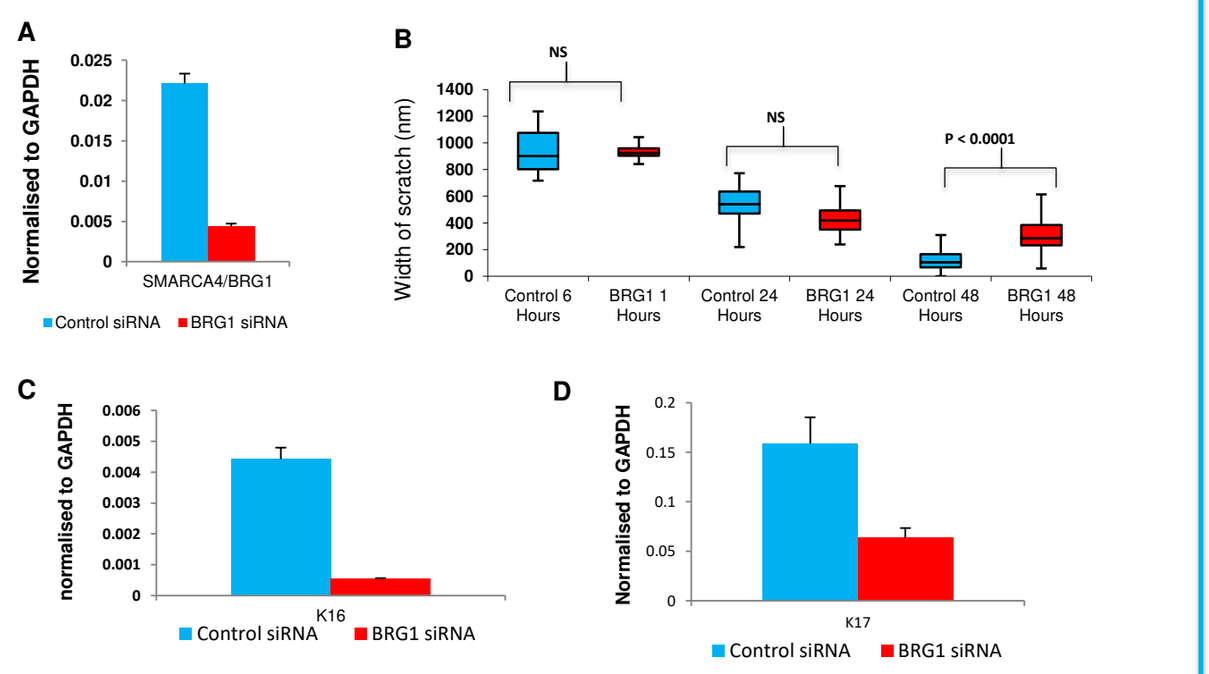
HaCaT keratinocytes were treated with either BRG1 siRNA or control siRNA for 48 hours, followed by treatmant with Mitomycin C and scratching to introduce in vitro wound and imaged in 1, 24 and 48 hours after the scratching.
A) qRT-PCR analysis demonstrated the efficient suppression of BRG1 mRNA expression in 48 hour post transfection and up to 96 hours post transfection (data not shown) with BRG1 siRNA in HaCaT keratinocytes.
B) Significant reduction in the rate of migration in BRG1 siRNA transfected HaCaT keratinocytes in comparison to the control siRNA. (Mann-Whitney U-test, 3 replicates per time point, 20 areas measured for each replicate).
C) siRNA mediated suppression of BRG1 did not change the percentage of Ki-67 positive HaCaT keratinocytes in comparison to controls.

Figure 4. BRG1 is required for the induction of wound associated cytokeratins 16 and 17



qRT-PCR analysis of RNA isolated from HaCaT keratinocytes transfected with BRG1 or control siRNA in 48 hours post scratch wounding with K16 (A) and K17 specific primers (B). Mean and standard deviation are shown for three biological replicates.

Figure 5. BRG1 controls primary human epidermal keratinocyte migration and cytokeratin 16 and 17 activation after wounding in vitro



PHEK were treated with either BRG1 siRNA or control siRNA for 48 hours, followed by Scratching to introduce in vitro wound and imaged in 1, 24 and 48 hours after the scratching RNA was isolated at 48 hours after the scratching.
A) qRT-PCR analysis demonstrated the efficient suppression of BRG1 mRNA expression after 48 hour transfection and up to 96 hours after transfection (data not shown) with BRG1 siRNA in PHEK. B) Significant reduction in the rate of migration in BRG1 siRNA transfected PHEK cells in comparison to the control siRNA. (Mann-Whitney U-test) (3 replicates per time point, 20 areas measured for each replicate).
C,D) qRT-PCR analysis demonstrating the suppression of BRG1 results in a failure to induce wound associated cytokeratins (K) 16 and 17 at 48 hours, expression of K16/K17 is comparable at hours (data not shown) while at 48 hours a sharp increase in both K16 and K17 is seen in control siRNA treated cells but not BRG1 siRNA treated PHEKs (3 replicates per a time point).

Conclusions

>>> Genes encoding most SWI/SNF complex subunits are expressed in human epidermal keratinocytes.

>>>BRG1 protein expression is increased in hyperproliferative and migrating keratinocytes after skin wounding in comparison to distal unaffected epidermis.

>>> BRG1 deficiency in human primary keratinocytes results in defects of scratch associated wound closure due to suppression of cell migration, but not proliferation

>>> BRG1 suppression results in a failure to induce wound associated cytokeratins 16 and 17 in human epidermal keratinocytes

Future work

>>>Define transcription targets of BRG1 in migrating human epidermal keratinocytes during cutaneous wound healing.

>>>Determine the role of BRG1 and SWI/SNF complexes in ex vivo human whole skin wound healing model