Appendix S1

METHODS

Co-immunoprecipitation and western blot

This was performed according to the manufacturer's protocol (Pierce co-immunoprecipitation (Co-IP) kit, ThermoFisher Scientific, UK). Normal skin fibroblasts and keloid fibroblasts were lysed in Co-IP Lysis Buffer, the cell debris was pelleted and the protein quantified through the bicinchoninic acid protein assay reagent kit (BCA; ThermoScientific, UK). 1 mg of each cell population lysate was pre-cleared by 1 hour incubation with non-specific agarose resin. For each receptor subunit, the primary antibody was covalently bound to an amine-reactive resin and incubated with cleared cell lysate over-night at 4°C. Input lysate was also incubated with negative control resin as per the manufacturer's protocol. Non-bound protein was removed, control and specifically bound complex-resin washed and proteins eluted. Immunoprecipitate was subjected to western blot. Blots from each Co-IP reaction were probed with anti-ErbB2, 3 and 4 antibodies and developed using nitro blue tetrazolium/S-bromo-4-chloroindoxyl phosphate (NBT/BCIP, 34042, ThermoFisher Scientific, UK). The western blots were analysed using ImageJ software, including only the area where the protein band was located and each quantification repeated three times to ensure accuracy.

For western blot alone, each sample was run on the same blot as its own internal control using GAPDH. In order to compare samples the fold change over control was used and the mean of these fold changes quantified and presented on the bar graphs.

In vitro *scratch assay*

Both normal skin and keloid fibroblasts were seeded into 6-well plates (3×10^5) and grown to confluence (~90%). Normal skin fibroblasts were treated with 50 ng/ml rhNRG1 for 24 h. Keloid fibroblasts were treated with 50 ng/ml rhNRG1 alone, 5nM ErbB2 siRNA alone or combined rhNRG1 and ErbB2 siRNA. Control wells treated with serum-free medium alone or negative control siRNA and were performed in tandem. Scratch wounds were introduced using a sterile 10 µl pipette tip, cells were washed to remove cell debris and then treated as described above. Six scratch wound were introduced into each well and experiments performed in duplicate. Images were taken at time 0 and marks made adjacent to the scratch beneath the plate surface as reference points. Final images were taken at 48 hrs and this image compared with the original scratch wound at time 0. Fibroblasts were fixed with 4% formaldehyde for 30 min, permeabilised with 0.1% triton X-100 (Sigma-Aldrich, UK) and stained with F-actin using rhodamine isothiocyanate 1:250 (Sigma-Aldrich, UK) and nuclei stained with 1:500 DAPI. Three non-overlapping fields were captured at 10× (inverted Olympus IX71) for each scratch, equating to a total of 54 measurements for each experimental condition ($n = 3$) for each of keloid and normal skin fibroblasts. The number of cells (defined as cells with nuclei) migrated into the wound site counted and presented as mean ± SEM.