

Expansion of stem/progenitor cells; the potential of small interfering RNA strategies.

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INTRODUCTION:

The ability to expand stem cell populations will play an important role in the application of stem/progenitor cell therapy and tissue engineering related therapies. This study investigated the use of siRNA strategies to see if a clinically useful amplification of targeted cell populations could be achieved. It is our hypothesis that knocking down gene triggers for differentiation will prolong the proliferative phase and thereby increase amplification. In particular, the study will investigate the effect of siRNA driven alkaline phosphatase, osteocalcin and collagen I (ALP, OC, Coll I) gene knockdown on osteoblast-like cell proliferation and differentiation.

METHODS:

This pilot study used the Human Fetal Osteoblasts (HFOB 1.19) cell line in an *in vitro* model of osteoblast proliferation and differentiation. Following optimisation of tissue culture transfection conditions, the HFOBs were transfected with siRNAs to the genes alkaline phosphatase, osteocalcin and collagen I, singly and in combination then cultured for 7 days. Outcome measures: The effects of transfection on proliferation was assessed by cell number (Coulter counter) and gene knockdown was confirmed using Real-Time PCR.

RESULTS:

An increase in cell number was associated with transfection of alkaline phosphatase and osteocalcin but not collagen I. (Fig. 1). This effect on cell number was associated with the knockdown of the related genes (Fig 2.)

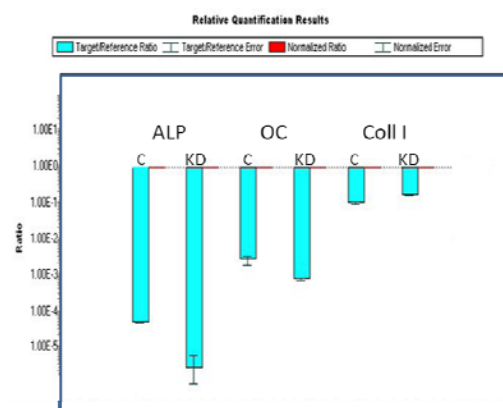
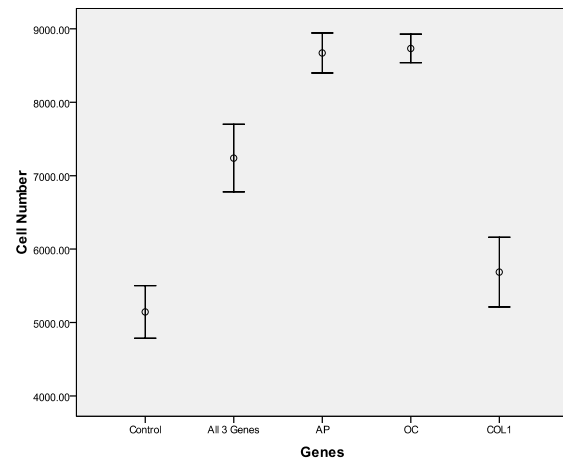


Fig.2 Effect of transfection on gene knockdown. Results are normalised to the B Actin housekeeping gene. C –control; KD - knockdown

DISCUSSION & CONCLUSIONS:

This pilot study provides evidence that transfection of osteoblast associated genes using siRNAs can delay differentiation/prolong proliferation in osteoblast-like cells.

These findings could have important implications for the targeted expansion of progenitor/stem cells in many organ systems and may provide the basis of a powerful tool for cell therapy.