

## HIGH-LEVEL EXPRESSION OF HUMAN GROWTH HORMONE (hGH) IN TRANSDUCED PRIMARY HUMAN KERATINOCYTES

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Primary human keratinocytes have been demonstrated to be suitable target cells for gene therapy. They are relatively easy to obtain and can easily be monitored for the expression of transgenes.

A retroviral vector encoding the human growth hormone gene (LhGHSN) was constructed and used to prepare ecotropic (GP+E+86) and amphotropic (GP+env AM12) producer cells lines. After the selection with G418, eighteen clones of the amphotropic cell line were expanded and the levels of hGH in the culture medium measured by RIA. Two of these clones, producing the highest hGH levels (216 and 188 ng/10<sup>6</sup> cells /day), were used to transduce primary human keratinocytes derived from the foreskin of normal children. Keratinocytes were seeded onto a feeder-layer composed of lethally irradiated 3T3 J2 cells and GP+env AM12 cells (1:2 mixture). The medium obtained from these transduced keratinocytes was collected and the hGH concentration was determined by RIA and Western blotting. In this medium the hGH concentration was about 4µg/10<sup>6</sup> cells/day while in the medium obtained from normal keratinocytes no presence of hGH was observed. By Western blot analysis only a 22 kDa protein, reacting with anti-hGH antibody, was found. This demonstrated the identity and integrity of secreted hGH.

The levels of hGH obtained in these experiments are the highest ever reported in the literature for primary human keratinocytes. Studies are in progress in order to carry out the grafting of epithelial sheets made with these transduced cells in different animal models.