Assessment of the *in vivo* Permanence of mGH-secreting Human Keratinocytes in Grafted Organotypic Cultures

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Keratinocytes are potential vehicles for delivering secreted gene products because these cells can be transferred to skin by relatively simple procedures of grafting. Previously, we constructed an efficient retroviral vector (LXSN) encoding the mouse growth hormone gene (mGH) that was used to transduce primary human keratinocytes. Organotypic raft cultures were prepared with these genetically modified keratinocytes and were grafted onto immunodeficient dwarf mice (*lit/scid*). mGH concentrations, determined by radioimmunoassay, revealed the highest *in vitro* expression and circulatory levels reported for a form of GH with this type of cells. Unfortunately these levels rapidly fell to baseline values. In the present study, a Northern bloting of mGH-transduced and non-transduced human keratinocytes, using a fragment of mGH cDNA as a probe, confirmed the clear presence of mGH mRNA. Immunohistochemical stainings based on anti-human involucrin, anti-human high molecular weight cytokeratin and anti-mouse growth hormone antibodies revelead the presence of human keratinocytes and mGH up to at least the seventh day after grafting. A Southern blot analysis confirmed the signal due to mGH cDNA in the excised implants. In conclusion, these results led us to analyse different factors involved in the persistence of the transgenic protein *in vivo*, such as the utilization of human keratinocytes and the influence of viral promoters. They also open new perspectives to study alternatives to this technique (use of mouse keratinocytes, different grafting procedures, naked DNA, etc) in view of clinical utilizations of this model of cutaneous gene therapy.

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