Enrichment of human keratinocyte stem cells and injection of naked DNA in the *lit/scid* mouse: studies directed to the improvement of GH gene therapy

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Gene therapy is not yet a routine in the clinic for treatment of systemic protein deficiencies. This is mainly due to problems in obtaining effective and sustained circulatory levels of the protein of interest. Several studies aiming at improving the methodology have mostly focused on growth hormone (GH) gene therapy (Peroni et al., *Current Gene Therapy*, 2005; 5: 493-509). Our work using human primary keratinocytes retrovirally transduced with the human (hGH) or the mouse (mGH) GH gene provided high *in vitro* expression levels, i.e. 7 μg hGH/10^6 cells/day and 11 μg mGH/10^6 cells/day. When these mGH-secreting keratinocytes were used in an organotypic raft culture model for grafting immunodeficient dwarf mice (*lit/scid*), the mGH levels in the circulation presented a peak value of ~20 ng/ml at 1 hour post implantation, followed by a rapid decline to baseline (~2 ng/ml) within 24 hours. Considering that transduction of stem cells seems to be a key requirement for sustained transgene expression *in vivo*, we experimentally determined that ~30 % of the cells in our keratinocyte population would be derived from transduced stem cells. Further some studies are now being carried out on the enrichment for keratinocytes stem cells with basis on their rapid attachment to a type of collagen. Another aspect related to the improvement of our cutaneous gene therapy model is the comparison of this *ex vivo* methodology with naked DNA injection of plasmids containing different promoters, such as β -actin, ubiquitin C or citomegalovirus. In a preliminar experiment in which our retroviral vector (based on a LTR promoter) was injected into *lit/scid* mice, unfortunately no hormone could be detected in the circulation. These studies, together with a determination of the human keratinocytes persistence in grafted mice, via an immunostaining methodology, are thus investigating the factors that still hamper a gene therapy clinical application for GH deficiency to become a realistic option for patients, in a near future.

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