P3.12. High-level Secretion of Growth Hormone by Retrovirally Transduced Primary Human Keratinocytes: Prospects for an Animal Model of Cutaneous Gene Therapy

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Gene therapy clinical trials for treatment of growth hormone (GH) deficiency has not been conducted yet, but several strategies using different gene transfer methodologies and animal models have been developed showing promising results. In our laboratory, we have set up an *ex vivo* gene therapy protocol using primary human keratinocytes transduced with an efficient retroviral vector (LXSN) encoding the human or the mouse GH (mGH) genes. These stably modified cells presented high *in vitro* expression levels of hGH (7 $\mu g/10^6$ cells/day) and mGH (11 $\mu g/10^6$ cells/day) after selection with geneticin. When the hGH-secreting keratinocytes were grafted onto immunodeficient dwarf mice (*lit/scid*), the hGH levels in the circulation did not fall below 0.2-0.3 ng/ml during a 12 day assay (peak value, 1.5 ng/ml at 4 h) and these animals presented a significant body weight increase ($P<0.01$) compared to the control *lit/scid* mice implanted with non-transduced keratinocytes (Bellini et al., FASEB J., 2003; 2322-2324). Epidermal sheets made with genetically modified keratinocytes, however, normally show a drop in secretion rates > 80 % due to detachment of the epithelium from its culture dish. Substitution of conventional grafting methodologies with organotypic raft cultures could completely overcome this problem. Grafts made with these cultured mGH-transduced cells revealed a relatively high peak value of up to 20 ng mGH/ml in the circulation of grafted *lit/scid* mice at 1 hour post-implantation, followed by a rapid decline to baseline (~2 ng/ml) within 24 hours. One week after grafting, however, the cultured excised implants recovered ~45 % of their original *in vitro* secretion efficiency. All these studies, including the determination of the percentage of transduced stem cells and the circulatory half-life of mGH and hGH in mice, are being carried out in order to identify the main factor(s) that still constitute one of the major impediments (i.e., sustainability of transgene secretion *in vivo*) to the success of this promising model of cutaneous gene therapy.

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