The feasibility of targeted selective gene therapy of the hair follicle

LINGNA LI & ROBERT M. HOFFMAN

AntiCancer Inc., 7917 Ostrow Street, San Diego, California 92111, USA
Correspondence should be addressed to R.M.H.

Loss of hair and hair colour is associated with ageing, and when it involves the scalp hair, it can be distressing to both sexes. Hair loss resulting from cancer chemotherapy is particularly distressing. However, safe, effective therapies directed to hair have only just started to be developed. The hair follicle is a complex skin appendage composed of epidermal and dermal tissue, with specialized keratinocytes, the hair matrix cells, forming the hair shaft. Specific therapy of the hair follicle depends on selective targeting of specific cells of the hair follicle. We have developed the histoculture of intact hair-growing skin on sponge-gel matrices[1-4]. We have recently found in histocultured skin that liposomes can selectively target hair follicles to deliver both small and large molecules[5,6]. That liposomes can target the hair follicle for delivery has been confirmed independently[7]. Two decades ago we introduced the technique of entrapping DNA in liposomes for use in gene therapy[8]. In this report we describe the selective targeting of the *lacZ* reporter gene to the hair follicles in mice after topical application of the gene entrapped in liposomes. These results demonstrate that highly selective, safe gene therapy for the hair process is feasible.

After topical application of liposome–*lacZ*, expression of the *lacZ* gene, indicated by blue staining of the X-gal substrate, was in the hair-forming hair matrix cells in the hair follicle bulbs (Fig. 1*a–c*) and in the bulge area (Fig. 1*d*, *e*) below the opening of the sebaceous gland, which is thought to contain the follicle stem cells[9]. The transfection frequency was high as many follicles are stained (Fig. 1*a*). No other cells were transfected with *lacZ* outside the follicle in the dermis or epidermis (Fig. 1*a*). Extensive *lacZ* expression in the hair matrix cells is shown (Fig. 1*b*, *c*). Transfection of what may be follicle stem cells also can be seen (Fig. 1*d*, *e*). Topical application of the naked *lacZ* gene did not result in gene transfer, and no LacZ staining was seen in follicles in animals not treated with liposome–*lacZ* (data not shown).

These results demonstrate that genes can be selectively targeted to the most important cells of the hair follicle, by liposomes representing the most selective targeting of a gene observed thus far *in vivo*[10]. This selectivity of gene targeting by topical liposome application suggests the feasibility of targeting hair matrix cells and possibly follicle stem cells to restore hair colour perhaps by delivery and expression of the tyrosinase gene[11,12] and with genes to restore hair growth. The highly selective nature of the topical application of liposome gene targeting lends itself to the development of practical and safe procedures.

Methods

Purification of *lacZ* DNA. A recombinant retrovirus containing the *lacZ* gene (pM-MuLV-SV-*lacZ*) was obtained from Joshua R. Sanes (Washington University, St. Louis, Missouri). The plasmid *lacZ* was transformed to HB101 *E. coli* competent cells (Promega) using standard procedures. The purification of plasmid *lacZ* DNA was obtained by using the Promega Wizard™ Megaprep DNA purification system.

Preparation of *lacZ*–liposomes. A total of 20 mg of lipid in a ratio of 5:3:2 of phosphatidylcholine:cholesterol:phosphatidylethanolamine

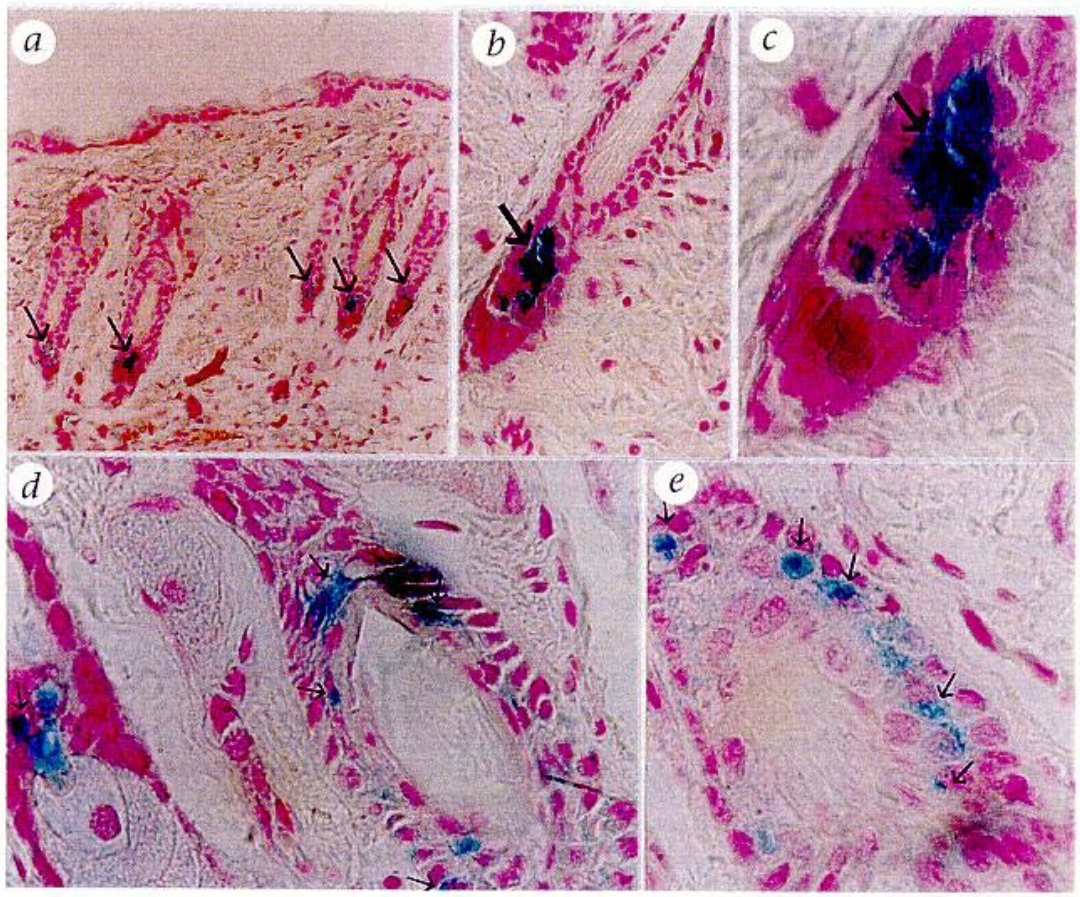

Fig. 1 Topical application of liposome–*lacZ* to mice. Blue stain indicates gene activity in the hair follicles after staining with the X-gal substrate. Note the selective delivery of the active gene to the hair matrix cells in the follicle bulbs (*a–c*) and to what may be follicle stem cells in the bulge area (*d, e*). Note the high frequency of transfection of *lacZ* gene in the hair follicles (*a*) and also total lack of gene activity outside the hair follicle (*a, b*). Nuclear fast red counter-staining. Light microscopy. Magnification, ×200 (*a*), ×400 (*b*) and ×1000 (*c–e*).

were rotary evaporated for 1 hour with a vacuum drier from a chloroform solution, to form a thin film on the wall of a 5-ml round-bottomed flask. The dried thin film lipid was suspended in 0.6-ml Tris-EDTA (TE) buffer containing approximately 1 mg of *lacZ* DNA on a vortex mixer. The *lacZ* DNA was entrapped by sonication in a compact water bath sonicator for 20 minutes, followed by freezing (at –70 °C) and thawing (at room temperature) three times.

Topical application of *lacZ*–liposomes. Preshaved 5- to 6-week-old BALB/c mice were used. The skin area for application of liposome-*lacZ* was prehydrated with phosphate-buffered saline (PBS) for 10–30 minutes. Liposome–*lacZ* formulation (50 µl) was placed directly on the skin with reapplication after 1 h. Untreated mice and mice treated with naked *lacZ* DNA were used as controls. The skin was carefully cleaned by 70% isopropyl alcohol before harvest for X-gal staining 3 days after application of the liposome–*lacZ* formulation.

Detection of *lacZ* DNA expression by X-gal staining. The harvested skin samples were immediately put into a modified Eagle's medium (MEM) wash containing a combination of antibiotics at 4 °C for 1 h and then fixed in 2% (vol/vol) formaldehyde-0.2% (vol/vol) glutaraldehyde in PBS for 30 minutes at 4 °C. Tissues were then rinsed with PBS three times and incubated in the X-gal staining solution containing 1 mg ml^{-1} X-gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and 2 mM $MgCl_2$ in PBS, at 37 °C for 18 h. Skin tissues were processed for paraffin sectioning by standard histological procedures and photographed under light microscopy after counter-staining with 0.1% nuclear fast red.

1. Li, L., Margolis, L.B. & Hoffman, R.M. Skin toxicity determined *in vitro* by three- dimensional, native-state histoculture. *Proc. natn. Acad. Sci. U.S.A.* **88**, 1908–1912 (1991).
2. Li, L., Margolis, L.B., Paus, R. & Hoffman R.B. Hair shaft elongation, follicle growth, and spontaneous regression in long-term, gelatin sponge-supported histoculture of human scalp skin. *Proc. natn. Acad. Sci. U.S.A.* **89**, 8764–8768 (1992).
3. Li, L., Margolis L.B., Lishko, V.K. & Hoffman, R.M. Product-delivering liposomes specifically target hair follicles in histocultured intact skin. *In Vitro cell. dev. Biol.* 28A, 679–681 (1992).
4. Li, L., Lishko, V.K. & Hoffman, R.M. Liposomes can specifically target entrapped melanin to hair follicles in histocultured skin. *In Vitro cell. dev. Biol.* 29A, 192–194 (1993).
5. Li, L., Lishko, V.K. & Hoffman R.M. Liposome targeting of high molecular weight DNA to the hair follicles of histocultured skin, A model for gene therapy of the hair growth processes. *In Vitro cell. dev. Biol.* 29A, 258–260 (1993).
6. Li, L., Lishko, V.K. & Hoffman, R.M. High efficiency liposome-mediated transfection of the tyrosinase gene to cultured cells: A model for the gene therapy of hair color restoration. *In Vitro cell. dev. Biol.* 30A, 135–138 (1994).
7. Yarosh, D. *et al.* Localization of liposomes containing a DNA repair enzyme in murine skin. *J. Invest. Dermatol.* 103, 461–468 (1994).
8. Hoffman, R.M., Margolis, L.B. & Bergelson, L.D. Binding and entrapment of high molecular weight DNA by lecithin liposomes. *FEBS Lett.* 93, 365–368 (1978).
9. Cotsarelis, G., Sun, T.T. & Lavker, R.M. Label-retaining cells reside in the bulge area of pilosebaceous unit: Implication for follicular stem cells, hair cycle and skin carcinogenesis. *Cell* 61, 1329–1337 (1990).
10. Caplen, N. J. *et al.* Liposome-mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Nature Med.* 1, 39–46 (1995).
11. Shibahara, S., Tomita, Y., Tagami, H., Muller, R.M. & Cohen T. Molecular basis for the heterogeneity of human tyrosinase. *J. exp. Med.* 156, 403–405 (1988).
12. Tanaka, S., Yamamoto, H., Takeuchi, S. & Takeuchi, T. Melanization in albino mice transformed by introducing cloned mouse tyrosinase gene. *Development* 108, 223–227 (1990).