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Letter to the Editor

MODEL OF SELECTIVE GENE THERAPY OF HAIR GROWTH: LIPOSOME TARGETING OF THE ACTIVE LAC-Z GENE TO HAIR FOLLICLES OF HISTOCULTURED SKIN

Dear Editor:

The histoculture of intact skin on sponge-gel matrices offers many opportunities for tissue engineering and the in vitro study of important processes intrinsic to skin such as hair growth and toxicity to various agents (Li et al., 1991, 1992a, 1992b, 1992c). We have recently exploited this system to demonstrate that liposomes can selectively target hair follicles to deliver small and large molecules including DNA.

Liposomes have been widely and successfully used as delivery systems to transport macromolecular substances that can not normally cross the plasma membrane into the cell (Gregoriadis 1984, 1988). We have recently reported that calcein-dye entrapped liposomes specifically targeted hair follicles in histocultured intact skin (Li et al., 1992d). Our results were strongly confirmed by studies by Lieb et al. (1992) demonstrating that liposomal formulations were more efficient than any other vehicle tested for delivery of carboxyfluorescein through the follicular route into the pilosebaceous units with the hamster ear model. We then developed liposome-mediated targeted delivery of melanin into hair follicles and the hair shaft itself in histocultured white-haired mouse skin (Li et al., 1993a). Recently, Yarosh also reported that liposomes allow T4 endonuclease V to penetrate into living skin (Yarosh et al., 1992). These studies have many ramifications including targeted hair growth modifications.

A number of years ago, we developed the technique of entrapping DNA in liposomes (Hoffman et al., 1978). We have utilized DNA liposomes in recent studies (Li et al., 1993b) to target high molecular weight DNA to the hair follicle itself as a model of gene therapy of the hair growth processes. In this report, we describe liposome targeting of the active bacterial Lac-Z gene to hair follicles of histocultured mouse skin.

A recombinant retrovirus containing the Lac-Z gene (pM-MuLV-SV-Lac-Z) was obtained from Dr. Joshua R. Sanes (Washington University Medical Center, St. Louis, MO). The Lac-Z plasmid was transfected into HB101 *E. coli* competent cells (Promega, Madison, WI) by standard methods. The purification of Lac-Z plasmid DNA was carried out with the Promega Wizard™ Maxiprep DNA purification system.

A total of 20 mg of lipid in a ratio of 5:3:2 of phosphatidylcholine (PC): cholesterol (Chol): phosphatidylethanolamine (PE) were rotary evaporated for 1 h 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 2 ml phosphate buffered saline (PBS, pH 7.4) on a vortex mixer and then sonicated with a Branson prob-type sonicator fitted with a microtip at power level 3, for 8 min. Then 300 μg of the plasmid Lac-Z DNA were entrapped in the above suspension by water bath sonication for 3 min, followed by freezing and thawing three times.

Pieces of outbred white-haired-mouse skin (approximately 2 × 5 × 2 mm) derived from 2–3 wk old animals were harvested under a dissection microscope and then histocultured on collagen-gel-supported sponges. Liposome interaction with the skin was initiated after 24 h of histoculture. Mouse skin histocultures were then incubated with liposome-Lac-Z for 4 d. A solution of naked-Lac-Z DNA at the same concentration that was used in the liposome preparation served as the control and was also incubated with skin histocultures.

The histocultured skin samples were first fixed in 2% (vol/vol) formaldehyde-0.2% (vol/vol) glutaraldehyde in PBS for 30 min at 4° C. Then the tissues were rinsed with PBS three times and incubated in the X-gal staining solution containing 1 mg/ml X-gal, 5 m*M* potassium ferricyanide, 5 m*M* potassium ferrocyanide, and 2 m*M* $MgCl_2$ in PBS, at 37° C for 18 h. Skin tissues were directly observed under light microscope.

Histocultured skin samples were then evaluated using light microscopy at 125× and 250× magnification. The results are shown in Fig. 1 *A–D*. The presence of the expressed Lac-Z gene, indicated by dark blue spots, is only seen in Fig. 1 *A* and 1 *B*, which received liposome-entrapped plasmid; no dark spots are observed in Fig. 1 *C*, and 1 *D*, which are the control treated with the naked Lac-Z gene. Furthermore, the dark spots are observed in the hair follicles and not significantly observable in the tissues adjacent to the hair follicles.

The results show that the liposome-targeted Lac-Z gene was expressed in hair follicles and was not detectable in the other portions of the histocultured skin sample, indicating the hair-follicle selectivity of the liposome delivery method. Future experiments will focus on targeting genes such as the tyrosinase gene into mouse hair follicles in vivo to restore hair color and other genes to restore hair growth.

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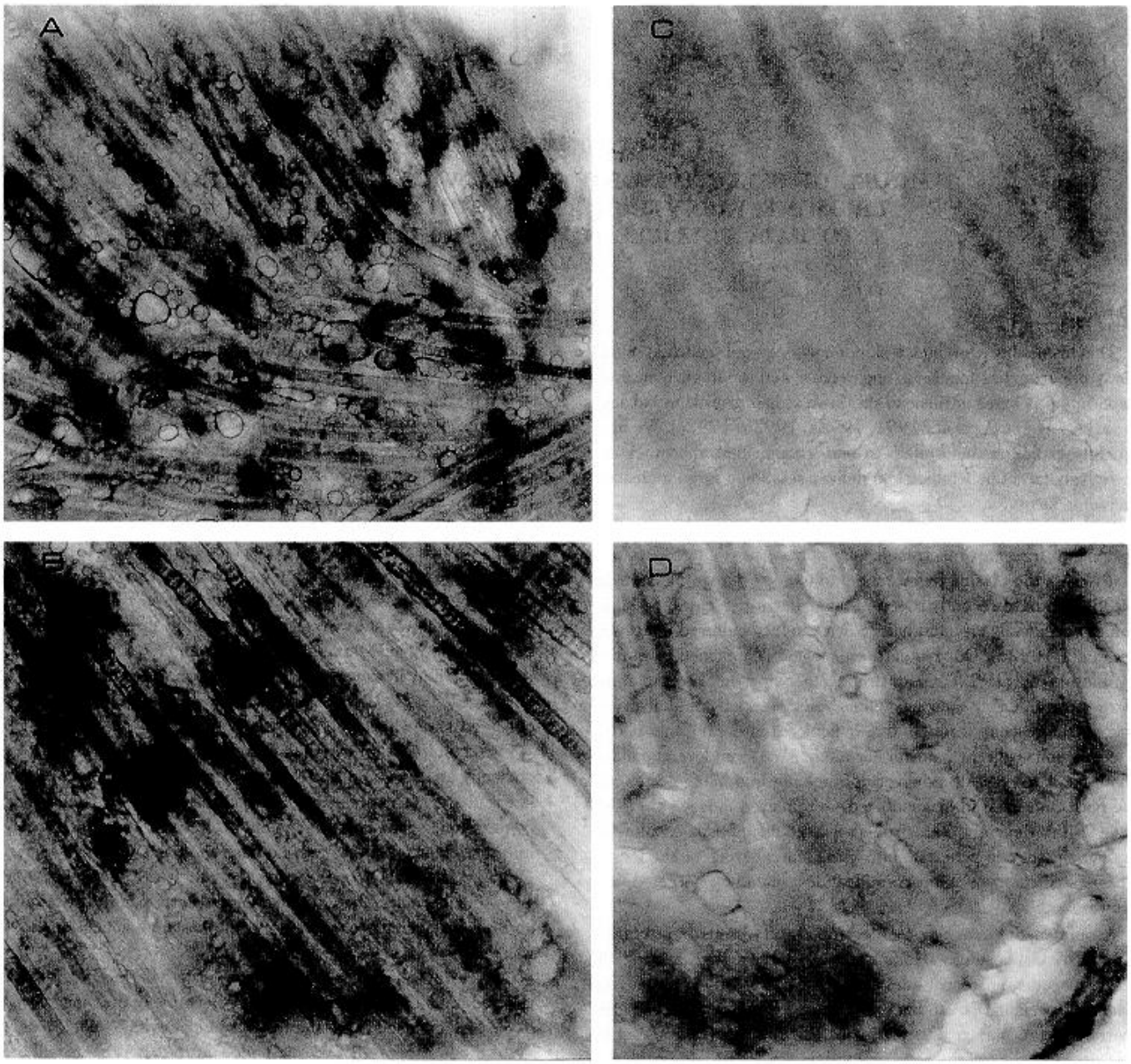

FIG. 1. (*A,B.*) Histocultures of white-haired mouse skin were treated with the liposome-entrapped Lac-Z gene for 4 d and X-gal stained for 18 h. *Note* the blue spots in the tissue and follicles and shafts indicating active gene transfer by the liposomes. Light microscopy. Magnification ×125 (*A*) and ×250 (*B*). (*C,D*). Control treated with the naked Lac-Z gene. *Note* that no transfected cells can be seen in the tissues. Magnification ×125 (*C*) and ×250 (*D*).

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Lingna Li Robert M. Hoffman

AntiCancer Inc. (L. L., R. M. H.)
7917 Ostrow Street
San Diego, California 92111; and
Laboratory of Cancer Biology (R. M. H.)
Department of Pediatrics, 0609F
University of California, San Diego
La Jolla, California 92093

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