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Letter to the Editor

HAIR GROWTH IN VITRO FROM HISTOCULTURED SKIN

Dear Editor:

The highly-regulated multicellular system supporting hair growth has been the subject of many investigations. As with other biological phenomena the study of many aspects of hair growth in controlled systems requires in vitro model systems. In recent decades many attempts to establish in vitro systems of hair growth were undertaken (e.g., Strangeways, 1931; Murray, 1935; Hardy, 1951; Frater and Whitmore, 1931; Philpott et al., 1989; and others). Successful hair growth in isolated human hair follicles free-floating in culture medium was reported by Philpott et al. (1990). In the present report we describe a new in vitro system of hair growth utilizing sponge-gel supported histocultures of intact mouse skin based originally on the early work of Leighton (1951) and on more recent work of Hoffman and his co-workers (Freeman and Hoffman, 1986; Vesico et al., 1987, 1990a,b; Li et al., 1991; Hoffman, 1991a–c; Baibakov et al., 1991; Guadagni et al., 1991; Furukawa et al., 1992; Guadagni et al., 1992). This histoculture system enables us to maintain histologically-intact specimens of human skin supported on collagen sponge gels for many days. Below we describe the growth of hair from specimens of mouse skin maintained in histoculture for up to 10 days.

Pieces of shaved outbred white-mouse skin (approx. 2 × 5 × 2 mm) were obtained under a dissecting microscope. The methods of culturing, [^{3}H]thymidine labelling, sectioning and preparing autoradiograms of skin were described earlier (Li et al., 1991).

Three types of sponge-gels for culture support were used: collagen-containing sponges, cellulose sponges or a combination of them. The viability of the cells in the specimens was determined by staining with the dyes bis-carboxyethylcarboxyfluorescein (BCECF) and propidium iodide (PI) that report live and dead cells respectively. The specimens were analyzed with a BioRad MRC-600 confocal scanning-laser microscope and Nikon fluorescence microscope as described by us earlier (Li et al., 1991).

Intensive hair growth was observed in the pieces of shaved mouse skin histocultured on the sponge-gel support. As seen in the Fig. 1 (*A–C*) the length of the hairs increased with days of incubation. This is summarized in the graph of Fig. 1 *D*, based on serial measurements of the length of hairs from two specimens in three sets of experiments. As Fig. 1 *D* demonstrates, the hair growth pattern in skin histocultures was similar to that in vivo. In some specimens hair growth was observed even on day 10, although as seen from Fig. 1 *D* growth is slowed down after the first 2 days in culture. Pieces of cultured skin either gave rise to at least 5 hairs or do not produce hair at all. Since all histocultures were treated similarly and in many cases different histocultures were maintained in the same medium, we attribute the failure to produce hair in some pieces of mouse skin to injury during the operation procedure.

The viability of the individual cells in the hair follicles was determined by the relative fluorescence of cells stained by the combination of BCECF and PI visualized by confocal microscopy. In this determination, the living cells are stained green by BCECF, while the dead cells are stained red by PI. As shown in Fig. 1 *E* even at Day-8 when hair growth is slowed there are only a few dead cells, while the rest remain alive as judged by the fluorescent dyes. The cells in the hair follicles are not only viable but also demonstrate an intensive DNA synthesis (Fig. 1 *F*) as determined by histological autoradiography of paraffin sections of specimens previously incubated with [^{3}H]thymidine.

To determine whether the hair-producing skin histocultures respond to chemotherapeutic drugs in a way similar to skin in vivo we have applied doxorubicin to the histocultures. This widely-used chemotherapeutic drug causes alopecia in patients. Doxorubicin at a concentration of 29 ng/ml completely prevents hair growth in one-day old cultures when applied for the next three days. The control cultures demonstrated the normal rate of hair growth. The average length of the hairs on day 4 in doxorubicin-treated cultures was .0 ± .0 versus 0.9 mm ± 0.16 in the control.

Thus the sponge-gel-supported histocultures of intact skin described above can be used to study the regulation of hair growth and can serve as an assay system for hair stimulators and inhibitors.

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References

Baibakov, B.; Frank, G. A.; Sergeeva, N., et al. *In vivo* growth patterns of human lung tumors in three-dimensional histoculture. In Vitro Cell. Dev. Biol. 27A:897–899; 1991.

Frater, R.; Whitmore, P. G. The *in-vitro* growth of post-embryonic hair. J. Invest. Dermatol. 61:72–81; 1973.

Freeman, A. E.; Hoffman, R. M. *In vivo*-like growth of human tumors *in vitro*. Proc. Natl. Acad. Sci. USA 83:2694–2698; 1986.

Furukawa, T.; Kubota, T.; Watanabe, M., et al. High *in vitro-in vivo* correlation of drug response using sponge-gel supported three-dimensional histoculture and the MTT end-point. Int. J. Cancer. In press.

Guadagni, F.; Li, L.; Hoffman, R. M. Targeting antibodies to live tumor tissue in 3-D histoculture. In Vitro Cell. Dev. Biol. 28A:297–299; 1992.

Guadagni, F.; Roselli, M.; Hoffman, R. M. Maintenance of expression of tumor antigens in three-dimensional in vitro human tumor gel-supported histoculture. Anticancer Res. 11:543–546; 1991.

Hardy, M. H. The development of pelage hairs and vibrissae from skin in tissue culture. Ann. NY Acad. Sci. 53:546–561; 1951.

Hoffman, R. M. In vitro sensitivity assays in cancer: a review, analysis and prognosis. J. Clin. Lab. Anal. 5:133–143; 1991a.

Hoffman, R. M. Three-dimensional gel-supported native-state histoculture for evaluation of tumor-specific pharmacological activity: principles, practices, and possibilities. J. Cell. Pharmacol. 2:189–201; 1991b.

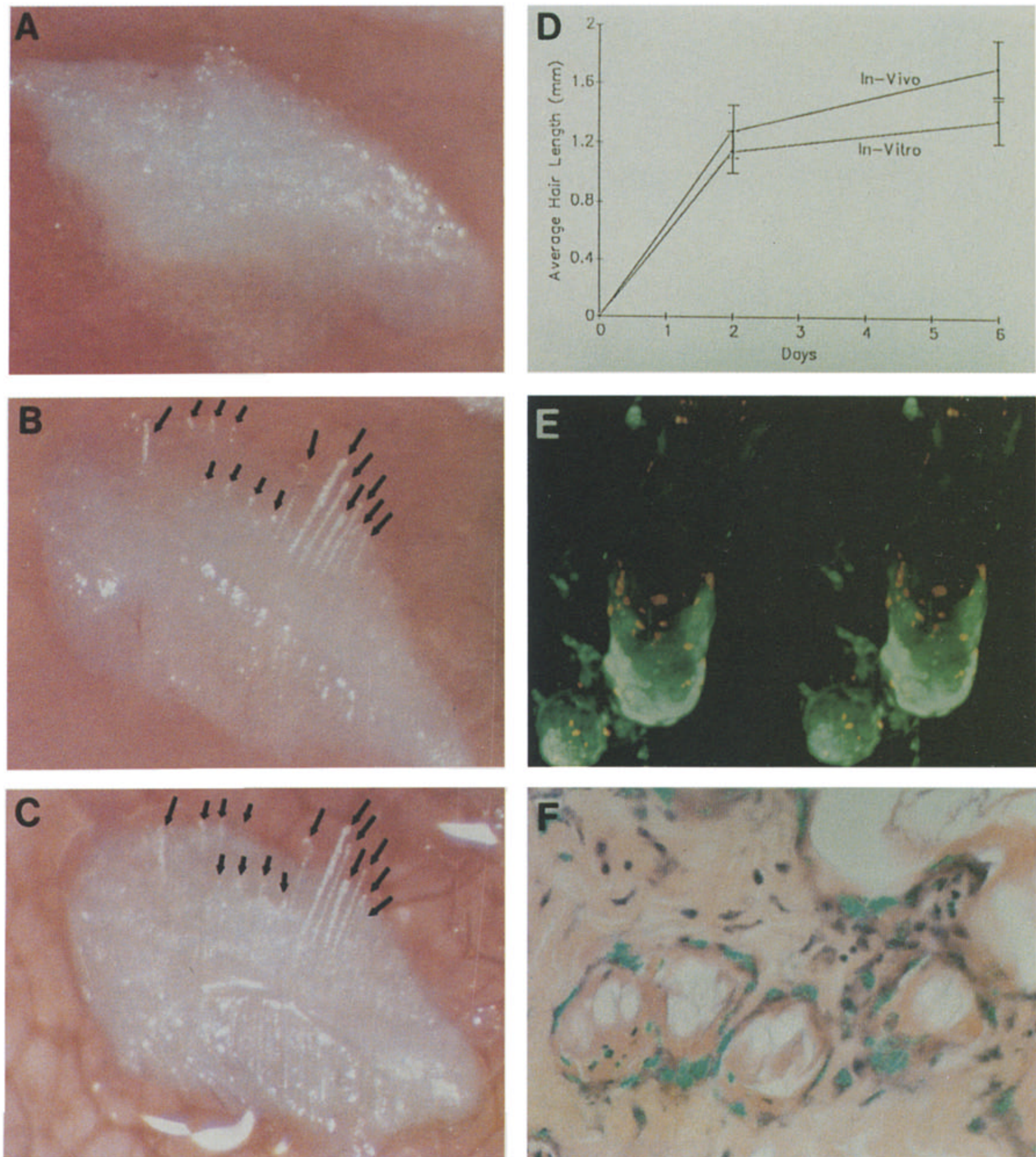

FIG. 1. *A*. Shaved-mouse skin histocultured on gelfoam-coated cellulose sponge at time of explantation. *Note* the absence of hair. Dissection microscopy. Magnification ×5.25. *B*. Same specimen as *A* after histoculturing on gelfoam-coated cellulose sponge for two days. *Note* the hair growing out from the skin. Dissection microscopy. Magnification ×5.25. *C*. Same specimen as *A* after histoculturing on gelfoam-coated cellulose sponge for 6 days. *Note* further hair growth. Dissection microscopy. Magnification ×5.25. *D*. Comparison of the pattern of hair growth in vitro and in vivo versus time. Hair growth in vitro has high correlation with in vivo hair growth. *E*. Hair follicles of mouse skin histocultured for 8 days and double-stained by BCECF-AM (*green, living*) and PI (*red, dead*). *Note* that most follicle cells are viable. Confocal scanning-laser microscopy. Magnification ×350. *F*. Autoradiography of paraffin section of shaved mouse skin histocultured for 6 days and labeled with [^{3}H]thymidine for 4 days. *Note* the high labeling of follicle cells (*bright green grains*). Polarized-light and bright-field microscopy. Magnification ×500.

Hoffman, R. M. Three-dimensional histoculture: origins and applications in cancer research. Cancer Cells 3:86–92; 1991c.

Hoffman, R. M.; Connors, K. M.; Meerson-Monosov, A. Z., et al. A general native-state method for determination of proliferation capacity of human normal and tumor tissue in vitro. Proc. Natl. Acad. Sci. USA 86:2013–2017; 1989.

Leighton, J. A sponge matrix method for tissue culture. Formation of organized aggregates of cells in vitro. J. Natl. Cancer Inst. 12:545–561; 1951.

Leighton, J. The Spread of Cancer. New York-London: Academic Press; 1967.

Li, L.; Margolis, L. B.; Hoffman, R. M. Skin Toxicity determined in vitro by three dimensional, native-state histoculture. Proc. Natl. Acad. Sci. USA 88:1908–1912; 1991.

Murray, M. R. Development of the hair follicle and hair in vitro. Anat. Rec. 57 (Supplement):74; 1933.

Philpott, M.; Green, M.; Kealey, T. Studies on the biochemistry and morphology of freshly isolated and maintained rat hair follicles. J. Cell Sci. 93:409–418; 1989.

Philpott, M.; Green, M.; Kealey, T. Human hair growth in vitro. J. Cell Sci. 97:463–471; 1990.

Strangeways, D. H. The growth of hair in vitro. Arch. Exp. Zellforsch. 11:344; 1931.

Vescio, R. A.; Connors, K. M.; Bordin, G. M., et al. The distinction of small cell and non-small cell cancer by growth in native-state histoculture. Cancer Res. 50:6095–6099; 1990.

Vescio, R. A.; Connors, K. M.; Youngkin, T., et al. Cancer biology for individualized therapy: correlation of growth fraction index in native-state histoculture with tumor grade and stage. Proc. Natl. Acad. Sci. USA 87:691–695; 1990.

Vescio, R. A.; Redfern, C. H.; Nelson, T. J., et al. In vivo-like drug responses of human tumors growing in three-dimensional, gel-supported, primary culture. Proc. Natl. Acad. Sci. USA 84:5029–5033; 1987.

Lingna Li
Ralf Paus
Leonid B. Margolis
Robert M. Hoffman

AntiCancer, Inc. (L. L., R. M. H., L. B. M.)
5325 Metro Street
San Diego, California 92110;
University Hospital (R. P.)
Rudolph Virchow
Freie Universitat Berlin;
Belozersky Institute (L. B. M.)
of Physico-Chemical Biology
Moscow State University,
Moscow, 119899, Russia; and
Laboratory of Cancer Biology (R. M. H., L. B. M.)
Department of Pediatrics, 0609F
University of California, San Diego
La Jolla, California 92093

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