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Mimicking hair disorders by genetic manipulation of organ cultured human hair follicles

Jiang Chen and **Dennis R. Roop**
Department of Dermatology and Charles C. Gates Center for Regenerative Medicine and Stem Cell Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Abstract

Human hair follicles can be dissected out of the scalp skin and cultured *in vitro* in defined growth medium. Hair follicle organ cultures have previously been used to investigate the molecular and cellular mechanisms through which various factors regulate the maintenance and cycling of adult hair follicles. In this issue, Samuelov *et al.* transfected organ-cultured human hair follicles with siRNA nucleotides and suppressed the expression of the endogenous P-cadherin gene in follicular keratinocytes. Knocking-down the expression of P-cadherin in hair follicles *in vitro* recapitulated the hair follicle phenotype observed in patients with hypotrichosis with juvenile macular dystrophy (HJMD), and enabled the authors to establish a cause-effect relationship between loss of P-cadherin and suppression of the canonical Wnt signaling pathway and, upregulation of TGFβ2 during the development of the hair abnormalities observed in HJMD patients.

Keywords

hair follicle; human; organ culture; *in vitro* model; genetic disorder

In vivo and *in vitro* models are valuable tools for hair research

Mouse hair follicles share a number of remarkable similarities with those of human, making the mouse a favorite *in vivo* model system for understanding the development and maintenance of human hair follicles, and dissecting the pathophysiology responsible for different hair disorders (Sundberg, 1994). Genetically engineered mutant mouse models developed by transgenic and gene targeting technologies have given us unprecedented opportunities for understanding the role of genes and signaling pathways in hair follicle morphogenesis and cycling, and they provide unique preclinical models for developing and testing new therapies for hair disorders (Schneider, 2012). Despite the fact that distantly related mammalian species share remarkably conserved molecular and cellular processes in hair follicle neogenesis and cycling (Zheng *et al.*, 2010), mouse models carrying analogous mutations underlying a human disorder do not always recapitulate the human hair phenotypes (Magerl *et al.*, 2004). This stems in part from differences in the anatomical and microscopic structure of human and mouse hair follicles, their genetic and epigenetic make-up and regulation, and their susceptibility and/or resistance to the development of certain

Correspondence: Jiang Chen, M.D., Department of Dermatology and Center for Regenerative Medicine and Stem Cell Biology, University of Colorado Anschutz Medical Campus, 12800 East 19th Avenue, RC-1 North, P18-8126, Mail Stop 8320, Aurora, CO 80045, USA, Phone: 303-724-5289, Fax: 303-724-3051, jiang.chen@ucdenver.edu.
Current address: Departments of Pathology and Dermatology, Stony Brook University School of Medicine, Basic Science Tower, Level 9, Room 151, Stony Brook, NY 11794, Phone: 631-444-3199, Fax: 631-444-3424, jiang.chen@stonybrookmedicine.edu

Conflict of Interest
None

skin and hair disorders. In addition, a single genetic modification made in the mouse can result in systemic or species-specific responses, which may be irrelevant to the corresponding phenotype in humans, and therefore, make the mouse model unreliable in understanding the corresponding human disorder. Thus, in certain circumstances, modeling human hair disorders may require a simpler system that is of human origin to facilitate signal read-out and to recapitulate the pathophysiology and damage response pathways involved in the development of human hair disorders.

The cultivation of adult human hair follicles, which was first reported in 1990 (Kondo *et al.*, 1990; Philpott *et al.*, 1990), represents an attractive *in vitro* model system for hair research. Using a straight forward micro-dissection technique, hair follicles with intact outer root sheath and dermal connective tissue sheath can be isolated from scalp skin (Philpott *et al.*, 1990) or other locations of the body (Kondo *et al.*, 1992), and subsequently cultivated *in vitro* as an organ culture (Fig. 1). The majority of scalp hair follicles are in anagen VI (Paus and Cotsarelis, 1999; Sinclair *et al.*, 1999), and are capable of sustaining follicular growth characterized by, among others, follicular matrix cell proliferation and hair shaft elongation (Philpott *et al.*, 1994). More importantly, cultured hair follicles can produce hair shafts at a rate close to that of normal hair growth *in vivo* (Myers and Hamilton, 1951). After a limited period of linear growth, *in vitro* cultured human hair follicles spontaneously stop growing and transform into catagen characterized by reduced matrix cell DNA synthesis and proliferation, increased apoptosis, reduced pigmentation, and cessation of hair follicle extension (Kloepper *et al.*, 2010). Given the fact that the anagen-catagen transition is one of the most clinically relevant events of the hair cycle that is associated with a wide range of hair growth disorders caused by premature anagen-catagen transition, for example, androgenic alopecia and telogen effluvium (Paus, 2008; Sinclair *et al.*, 1999), organ cultured scalp hair follicles represent a simple, cost effective, and instructive model system for investigating the pathophysiology of hair loss.

Cultivating hair follicles in defined medium (Williams E medium supplemented with 2 mM L-glutamine, 10 μg/ml insulin, 10 ng/ml hydrocortisone, 100 U/ml penicillin and 100 μg/ml streptomycin, without serum), has enabled researchers to characterize a long list of factors known to be important for hair growth, including: growth factors, hormones, cytokines, immune modulators, vitamins, drugs used in chemotherapy, environmental stimuli, and numerous natural substances (Randall *et al.*, 2003; Rogers and Hynd, 2001; Yoon, 2009). While previous reports were based on the study of exogenous growth modulators which were supplemented into the growth medium (Randall *et al.*, 2003; Rogers and Hynd, 2001; Yoon, 2009), the genetic manipulation of endogenous genes known to be essential for the maintenance and cycling of adult hair follicles has not been reported. In this issue, Samuelov *et al.* report the genetic manipulation of organ cultured human hair follicles through the silencing of an endogenous gene in follicular keratinocytes.

Genetic manipulation of organ cultured human hair follicles

Hypotrichosis with juvenile macular dystrophy (HJMD) is an autosomal recessive disorder caused by loss-of-function mutations in the *CDH3* gene (Sprecher *et al.*, 2001). To mimic the development of hair follicle abnormalities observed in HJMD, Samuelov *et al.* utilized an organ culture model consisting of scalp hair follicles in which expression of the endogenous P-cadherin gene (*CDH3*) was knocked down with siRNA oligonucleotides. Of special interest, the hair phenotype associated with HJMD was not observed in mice with a germline deletion of P-cadherin (Radice *et al.*, 1997).

CDH3 siRNA oligonucleotides were delivered to the organ cultured hair follicles by lipofectamine. Remarkably, 24 hours after siRNA delivery (2 days of *in vitro* culture), the

transcription levels of *CDH3* diminished to a level comparable to that of catagen hair follicles cultured *in vitro* for 8 days; thus, recapitulating the loss of P-cadherin in HJMD hair follicles at the transcriptional level. The authors demonstrate that loss of *CDH3* in follicular keratinocytes triggered a pre-mature anagen-catagen transition characterized by reduced proliferation of matrix cells, reduced growth of the hair shaft and production of hair keratins, suppressed signaling via the canonical Wnt pathway, and increased TGFβ2 signaling. Therefore, genetic manipulation of organ cultured human hair follicles allowed the authors to establish a cause-effect relationship between the loss of P-cadherin and the suppression of canonical Wnt signaling during the development of hair loss in HJMD.

This report highlights the possibility of using *in vitro* cultured human hair follicles to recapitulate the genetic and phenotypic abnormalities observed in other human hair disorders, and thus potentially decreasing the lag time between laboratory discoveries and clinical translation. To further validate this *in vitro* model for the future development of therapeutic strategies for HJMD, it would be of interest to determine the feasibility of culturing hair follicles from HJMD patients, and testing whether the restoration of P-cadherin expression, or its downstream effectors, would extend anagen VI and delay the onset of catagen.

Future development of organ cultured human hair follicles as models to study human hair disorders

Although the report of Samuelov *et al.* is an excellent example of the advantages in using *in vitro* cultured human hair follicles, there are obvious deficiencies in this model that may limit its use for other applications. For example, *in vitro* cultured human hair follicles lack a native vasculature system, a neuro-endocrine supply, an intact immune system, and the influence of the surrounding micro- and macro-environment, and global regulators. Therefore, organ cultured hair follicles are not *bona fide* hair follicles *in vivo*. Consequently, organ cultured human hair follicles are unable to maintain normal cycling, and may differ from *in vivo* hair follicles in their response to stimuli, a case in point, the inconsistent findings in recapitulating the clinical benefit of minoxidil (Kwon *et al.*, 2006; Magerl *et al.*, 2004; Miranda *et al.*, 2010). Only a few studies have evaluated putative drugs for their hair growth-promoting effects; the majority of previous studies have focused on the inhibitory effects of various agents on organ-cultured hair follicles (Randall *et al.*, 2003; Rogers and Hynd, 2001; Yoon, 2009), which was due to the inevitable transition of these organ cultured anagen VI hair follicles to catagen. Therefore, improved organ culture conditions capable of sustaining long-term hair follicle growth is highly desirable so that candidate therapeutic (anagen promoting) agents can be evaluated with organ cultured hair follicles.

Progress has been made in extending the duration of hair follicle growth *in vitro*, such as maintaining hair follicles *in situ* in organ cultured skin (Li *et al.*, 1992; Lu *et al.*, 2007). Organ cultures of scalp skin significantly extended the growth phase of hair follicles in serum-free medium at the air-liquid interface (Li *et al.*, 1992; Lu *et al.*, 2007). Organ cultures also preserved the entire pilosebaceous unit, including the bulge, and the microenvironment and neighboring follicles, enabling the possibility of examining stem cell activation, the influence of neighboring hair follicles, and the involvement of resident melanocytes and mast cells in hair follicle maintenance and cycling.

Can organ cultured hair follicles be used as a source of autologous hair follicles to treat hair loss?

The aim of treating hair loss is to prolong anagen or facilitate anagen re-entry (i.e. to resume normal hair cycling) through the use of drugs or natural substances. In cases where hair loss

is caused by mutations in known genes, the efficient genetic manipulation of hair follicles in organ cultures, as demonstrated herein and previously by Tiede et al. (Tiede *et al.*, 2010; Tiede *et al.*, 2009), offers the possibility of using a genetic approach to correct these genetic abnormalities in follicular keratinocytes or dermal papilla cells in organ cultured hair follicles isolated directly from patients. The genetic manipulation of organ cultured hair follicles would potentially result in a permanent corrective therapy prior to autotransplantion. Thus, the approach taken by Samuelov *et al.* has not only provided new insight into the pathophysiology underlying HJMD, but also given us a glimpse at a future therapeutic approach for correcting this and other genetic disorders that result in hair loss.

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Clinical Implications

- Human scalp hair follicles can be dissected out of the skin, and readily maintained in organotypic culture as surrogate mini-organs.
- Genetic manipulation can be achieved by transfecting cultured hair follicles to express exogenous genes or silence endogenous genes.
- Organ cultured hair follicles or skin from healthy individuals or patients can be used as a model to investigate disease development and progression, and to test novel therapies.

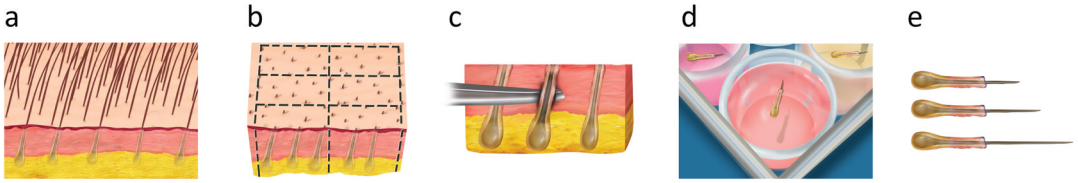

Fig. 1.

Human hair follicle isolation and organ culture. **a**, Scalp skin biopsy. **b**, Skin biopsy is cut into dissectible pieces. **c**, Distal portion of anagen hair follicles are removed and extra subcutaneous fat trimmed before hair follicles are dissected under stereo microscope. **d**, Dissected human hair follicles are cultured in 24-well plate. **e**, Cultured hair follicles grow *in vitro* and their length can be measured with the measuring graticule attached to the eyepiece of a microscope.