Expert Opinion



Cell- & Tissue-based Therapy

# The potential of nestin-expressing hair follicle stem cells in regenerative medicine

Robert M Hoffman
[†1]*AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111, USA, and*
[2]*University of California, San Diego, Department of Surgery, San Diego, California, USA*

The hair follicle bulge area is an abundant, easily accessible source of actively growing pluripotent adult stem cells. Nestin, a protein marker for neural stem cells, is also expressed in follicle stem cells and their immediate, differentiated progeny. Green fluorescent protein (GFP), whose expression is driven by the nestin regulatory element in transgenic mice, serves to mark hair follicle stem cells. The pluripotent nestin-driven GFP stem cells are positive for the stem cell marker CD34, but negative for keratinocyte marker keratin 15, suggesting their relatively undifferentiated state. These cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells and melanocytes *in vitro*. *In vivo* studies show that nestin-driven GFP hair follicle stem cells can differentiate into blood vessels and neural tissue after transplantation to the subcutis of nude mice. Hair follicle stem cells implanted into the gap region of a severed sciatic or tibial nerve greatly enhance the rate of nerve regeneration and the restoration of nerve function. The follicle cells transdifferentiate largely into Schwann cells, which are known to support neuron regrowth. The transplanted mice regain the ability to walk normally. Thus, hair follicle stem cells provide an effective, accessible, autologous source of stem cells for treatment of peripheral nerve injury.

Keywords: hair follicle stem cell, nestin, regenerative medicine

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## 1. The hair follicle and its stem cells

The hair follicle produces a terminally differentiated keratinized end product, the hair shaft, which is eventually shed. The follicle undergoes cyclical regeneration with at least eight different epithelial lineages [1]. Hair is formed by rapidly proliferating matrix keratinocytes in the bulb located at the base of the growing (anagen) follicle. The duration of anagen varies greatly between hairs of differing lengths. Nevertheless, matrix cells eventually stop proliferating and hair growth ceases at catagen when the lower follicle regresses (telogen). After telogen, the lower hair-producing portion of the follicle regenerates, starting the new anagen phase [1].

Hair follicle stem cells, locted in the hair follicle bulge, possess stem cell characteristics, including multipotency, high proliferative potential and ability to enter quiescence. Lineage analysis has demonstrated that all epithelial layers within the adult follicle and hair originate from bulge cells [1,2]. Therefore, the hair follicle stem cells appear to be responsible for regenerating the hair follicle in each hair cycle.



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## 2. Markers for hair follicle stem cells

The lack of markers to identify hair follicle stem cells in the bulge area has hindered the study of hair follicle stem cells. CD34 expression, as first defined by Trempus *et al.* [3], is a marker for hair follicle stem cells. Antibodies recognizing CD34 were used to collect viable bulge cells by fluorescence-activated cell sorting [3,4]. Keratin 15 (K15) is expressed at high levels in the bulge, but lower levels of expression can be present in the basal layers of the lower follicle outer root sheath and the epidermis [5,6]. A K15 promoter used for the generation of transgenic mice possesses a pattern of activity restricted to the bulge in the adult mouse [7]. Using transgenic mice in which the neural stem cell marker nestin was linked to green fluorescent protein (GFP), the author's group observed that nestin was also a marker for hair follicle stem cells [8]. The relatively small, oval-shaped, nestin-expressing cells in the bulge area surround the hair shaft and are interconnected by short dendrites. In mid and late anagen, the GFP-expressing cells are located in the upper outer root sheath, as well as in the bulge area, but not in the hair matrix bulb. These observations show that the nestin-expressing cells form the outer root sheath. Following the author's report that nestin-driven GFP (ND-GFP) can serve as a marker for hair follicle stem cells, Morris *et al.* [9] used GFP to isolate hair follicle stem cells in transgenic mice. Fuchs' group [10,11] also used GFP to identify hair follicle stem cells and possibly other skin stem cells in trasngenic mice. Recently, Yu *et al.* [12] showed that nestin was present in human hair follicle stem cells.

The evidence that nestin-expressing cells in the hair follicle bulge are hair follicle stem cells (rather than a population of stem cells that reside in the hair follicle whose purpose is to regenerate the neuronal and endothelial components associated with the pilosebaceous units), is that the nestin-expressing (and GFP-expressing) cells have been imaged over time to regenerate a large portion of the hair follicle [8].

The author's group have shown that nestin expression in hair follicle stem cells colocalizes with other follicular stem cell markers [8], but have not yet shown that the nestin-expressing cells are label-retaining, a feature of at least some stem cells.

## 3. Pluripotency of hair follicle stem cells

Hair follicle stem cells from adult mice, when combined with neonatal dermal cells, form hair follicles after injection into immunodeficient mice [4,9]. Cultured, individually cloned bulge cells from adult mice were also shown to form hair follicles in skin reconstitution assays [4].

Taylor *et al.* [13] reported that hair follicle bulge stem cells are potentially bipotent as they can give rise to not only cells of the hair follicle, but also epidermal cells. Other experiments [14] have also provided new evidence that the upper outer root sheath of vibrissal (whisker) follicles of adult mice contain multipotent stem cells, which can differentiate into hair follicle matrix cells, sebaceous gland basal cells and epidermis. Toma *et al.* [15] reported that multipotent adult stem cells isolated from mammalian skin dermis, termed skin-derived precursors, can proliferate and differentiate in culture to produce neurons, glia, smooth muscle cells and adipocytes. However, the exact location of these stem cells in skin is unknown and their functions are still unclear.

## 4. Differentiation of hair follicle stem cells to blood vessels

The author's group subsequently observed that ND-GFP in the transgenic mice also labels developing skin blood vessels that appear to originate from hair follicles and form a follicle-linking network. This was seen most clearly by transplanting ND-GFP-labeled vibrissa (whisker) hair follicles into unlabeled nude mice. New vessels grow from the transplanted follicle, and these vessels increase when the local recipient skin is wounded. The ND-GFP-expressing structures are blood vessels because they display the characteristic endothelial-cell-specific markers CD31 and von Willebrand factor [16].

## 5. Differentiation of hair follicle stem cells to neurons and other cell types

The author's group then demonstrated that ND-GFP hair follicle stem cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells and melanocytes *in vitro*. These pluripotent ND-GFP stem cells are positive for the stem cell marker CD34 and negative for K15, suggesting their relatively undifferentiated state. The apparent primitive state of the ND-GFP stem cells is compatible with their pluripotency. Furthermore, the author's group showed that the hair follicle stem cells differentiated into neurons after transplantation to the subcutis of nude mice [17].

## 6. Nerve cell repair using hair follicle stem cells

When the GFP hair follicle stem cells were implanted into the gap region of a severed sciatic nerve, they greatly enhanced the rate of nerve regeneration and the restoration of nerve function. The hair follicle stem cells differentiated largely into Schwann cells, which are known to support neuron regrowth. Function of the rejoined sciatic nerve was measured by contraction of the gastrocnemius muscle following electrical stimulation. The transplanted mice recovered the ability to walk normally [18].

## 7. Expert opinion

The hair follicle bulge area is an abundant, easily accessible source of actively growing pluripotent adult stem cells. The hair follicle stem cells express the stem cell markers CD34 and nestin, but are negative for the keratinocyte marker K15, suggesting their relatively undifferentiated state. These cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells and melanocytes *in vitro*. *In vivo* studies show ND-GFP hair follicle stem cells can differentiate into blood vessels and neural tissue after transplantation into the subcutis of nude mice. Hair follicle stem cells implanted into the gap region of a severed sciatic or tibial nerve greatly enhance the rate of nerve regeneration and the restoration of nerve function. The follicle cells transdifferentiate largely into Schwann cells, which are known to support neuron regrowth. The transplanted mice regain the ability to walk normally. Thus, hair follicle stem cells provide an effective, accessible, autologous source of stem cells for treatment of peripheral nerve injury. Thus, the hair follicle stem cells have potential as an alternative to the use of embryonic stem cells or fetal cells for regenerative medicine. The hair follicle stem cells do not have the ethical problems that embryonic or fetal stem cells have for use in regenerative medicine. Even more importantly, the hair follicle stem cells are more easily accessible than these other cells types and offer the potential for autologous treatment as they can be readily expended in culture after isolation from the patient.

It is also important to note that the dermal papilla is a potential source of multipotent stem cells that may have use in regenerative medicine. For example, Jahoda and collegues have demonstrated that hair follicle dermal cells repopulate the mouse haematopoietic system [19], can differentiate into adipogenic and osteogenic lineages [20], and participate in wound healing and induction [21].

Hair follicle stem cells also have great potential for hair resoration [1], but this topic is not in the scope of this review, which focusses on the use of hair follicle stem cells for regenerative medicine.

## Bibliography


1. COTSARELIS G: Epithelial stem cells: a folliculocentric view. *J. Invest. Dermatol.* (2006) 126:1459-1468.
2. COTSARELIS G, SUN TT, LAVKER RM: Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* (1990) 61:1329-1337.
3. TREMPUS C, MORRIS R, BORTNER C *et al.*: Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. *J. Invest. Dermatol.* (2003) 120:501-511.
4. BLANPAIN C, LOWRY WE, GEOGHEGAN A, POLAK L, FUCHS E: Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* (2004) 118:635-648.
5. LYLE S, CHRISTOFIDOU-SOLOMIDOU M, LIU Y, ELDER DE, ALBELDA S, COTSARELIS G: The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. *J. Cell Sci.* (1998) 111:3179-3188.
6. WASEEM A, DOGAN B, TIDMAN N *et al.*: Keratin 15 expression in stratified epithelia: downregulation in activated keratinocytes. *J. Invest. Dermatol.* (1999) 112:362-369.
7. LIU Y, LYLE S, YANG Z, COTSARELIS G: Keratin 15 promoter targets putative epithelial stem cells in the hair follicle bulge. *J. Invest. Dermatol.* (2003) 121:963-968.
8. LI L, MIGNONE J, YANG M *et al.*: Nestin expression in hair follicle sheath progenitor cells. *Proc. Natl. Acad. Sci. USA* (2003) 100:9958-9961.
9. MORRIS RJ, LIU Y, MARLES L *et al.*: Capturing and profiling adult hair follicle stem cells. *Nat. Biotechnol.* (2004) 22:411-417.
10. TUMBAR T, GUASCH G, GRECO V *et al.*: Defining the epithelial stem cell niche in skin. *Science* (2004) 303:359-363.
11. RHEE H, POLAK L, FUCHS E: Lhx2 maintains stem cell character in hair follicles. *Science* (2006) 312:1946-1949.
12. YU H, FANG D, KUMAR SM *et al.*: Isolation of a novel population of multipotent adult stem cells from human hair follicles. *Am. J. Pathol.* (2006) 168:1879-1888.
13. TAYLOR G, LEHRER MS, JENSEN PJ, SUN TT, LAVKER RM: Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell* (2000) 102:451-461.
14. OSHIMA H, ROCHAT A, KEDZIA C, KOBAYASHI K, BARRANDON Y: Morphogenesis and renewal of hair follicles from adult multipotent stem cells. *Cell* (2001) 104:233-245.
15. TOMA JG, AKHAVAN M, FERNANDES JL *et al.*: Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat. Cell Biol.* (2001) 3:778-784.
16. AMOH Y, LI L, YANG M *et al.*: Nascent blood vessels in the skin arise from nestin-expressing hair follicle cells. *Proc. Natl. Acad. Sci. USA* (2004) 101:13291-13295.
17. AMOH Y, LI L, KATSUOKA K, PENMAN S, HOFFMAN RM: Multipotent nestin-positive, keratin-negative hair-follicle-bulge stem cells can form neurons. *Proc. Natl. Acad. Sci. USA* (2005) 102:5530-5534.
18. AMOH Y, LI L, CAMPILLO R *et al.*: Implanted hair follicle stem cells form Schwann cells that support repair of severed peripheral nerves. *Proc. Natl. Acad. Sci. USA* (2005) 102:17734-17738.
19. LAKO M, ARMSTRONG L, CAIRNS PM, HARRIS S, HOLE N, JAHODA CA: Hair follicle dermal cells repopulate the mouse haematopoietic system. *J. Cell Sci.* (2002) 115(Pt 20):3967-3974.
20. JAHODA CA, WHITEHOUSE J, REYNOLDS AJ, HOLE N: Hair follicle dermal cells differentiate into adipogenic and osteogenic lineages. *Exp. Dermatol.* (2003) 12(6):849-859.
21. GHARZI A, REYNOLDS AJ, JAHODA CA: Plasticity of hair follicle dermal cells in wound healing and induction. *Exp. Dermatol.* (2003) 12(2):126-136.


## Affiliation


Robert M Hoffman[1,2] PhD
[†]Address for correspondence
[†1]AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111, USA
Tel: +1 858 654 2555; Fax: +1 858 268 4175; E-mail: all@anticancer.com
[2]University of California, San Diego, Department of Surgery, San Diego, California, USA